Renewable and Sustainable Energy Reviews 15 (2011) 4080–4106

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Progress in the production and application of n-butanol as a biofuel

Chao Jin[a,b], Mingfa Yao[c,*], Haifeng Liu[c], Chia-fon F. Lee[d,e], Jing Ji[b]

[a] School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[b] School of Agriculture and Bioengineering, Tianjin University, Tianjin 300072, China
[c] State Key Laboratory of Engines, Tianjin University, Tianjin 300072, China
[d] Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, IL 61801, USA
[e] Center for Combustion Energy and State Key Laboratory of Automotive Safety and Energy, Tsinghua University, Beijing 100084, China

ARTICLE INFO

Article history:
Received 13 December 2010
Accepted 24 June 2011
Available online 21 August 2011

Keywords:
Butanol
Biofuel
Fermentative production
Gasoline
Diesel fuel

ABSTRACT

Butanol is a very competitive renewable biofuel for use in internal combustion engines given its many advantages. In this review, the properties of butanol are compared with the conventional gasoline, diesel fuel, and some widely used biofuels, i.e. methanol, ethanol, biodiesel. The comparison of fuel properties indicates that n-butanol has the potential to overcome the drawbacks brought by low-carbon alcohols or biodiesel. Then, the development of butanol production is reviewed and various methods for increasing fermentative butanol production are introduced in detailed, i.e. metabolic engineering of the Clostridia, advanced fermentation technique. The most costive part of the fermentation is the substrate, so methods involved in renewed substrates are also mentioned. Next, the applications of butanol as a biofuel are summarized from three aspects: (1) fundamental combustion experiments in some well-defined burning reactors; (2) a substitute for gasoline in spark ignition engine; (3) a substitute for diesel fuel in compression ignition engine. These studies demonstrate that butanol, as a potential second generation biofuel, is a better alternative for the gasoline or diesel fuel, from the viewpoints of combustion characteristics, engine performance, and exhaust emissions. However, butanol has not been intensively studied when compared to ethanol or biodiesel, for which considerable numbers of reports are available. Finally, some challenges and future research directions are outlined in the last section of this review.

Contents

Abbreviations: ABE, Acetone butanol ethanol; ASOI, After start of injection; ATDC, After top dead center; BSFC, Break specific fuel consumption; BTDC, Before top dead center; CAD, Crank angle degree; CAI, Controlled autoignition; CFD, Computational fluid dynamics; CFR, Cooperative fuel research; CI, Compression ignition; CN, Cetane number; CO, Carbon monoxide; COV, Coefficient of variation; CVC, Constant volume chamber; DI, Direct-injection; EGR, Exhaust gas recirculation; HCCI, Homogeneous charge compression ignition; IMEP, Indicated mean effective pressure; ISFC, Indicated specific fuel consumption; JSR, Jet stirred reactor; LTC, Low temperature combustion; LTHR, Low temperature heat release; MBT, Maximum break torque; MFB, Mass fraction burn; MON, Motor octane number; NO_x, Nitrogen oxides; PAH, Polycyclic aromatic hydrocarbon; PM, Particulate matter; PON, Pump octane number; RCM, Rapid compression machine; RON, Research octane number; RPM, Revolution per minute; SI, Spark ignition; SOI, Start of injection; THC, Total hydrocarbon; UTG, Unleaded test gasoline.

* Corresponding author. Tel.: +86 22 27406842x8014; fax: +86 22 27383362.
E-mail addresses: jinchao@tju.edu.cn (C. Jin), y_mingfa@tju.edu.cn (M. Yao), haifengliu@tju.edu.cn (H. Liu), cflee@illinois.edu (C.-f.F. Lee), jijing@tju.edu.cn (J. Ji).

1364-0321/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rser.2011.06.001

1. Introduction

Biofuels are receiving increasing public and scientific attention, driven by factors such as uncertainties related to oil price, greenhouse gas emission, and the need for increased energy security and diversity. Biofuels are a wide range of fuels which are in some way derived from biomass [1]. It is reported that fossil fuels – oil, coal, and natural gas – dominated the world energy economy, covering more than 80% of the total primary energy supply [2]. Renewable energy sources accounted for 9.8% of the world's total primary energy supply in 2007, as shown in Table 1. Even for the 9.8% renewable energy, approximately two-thirds of biomass was used for cooking and heating because of its widespread noncommercial use in developing countries. The remaining one-third of biomass energy was utilized both in industrial applications within the heat, power, and road transportation sectors and for heating purposes in the private sector in industrialized countries. Actually, only about 2% in total global transport consumption was from the biofuels produced from biomass. Therefore, there is a large potential for the biofuels in the transportation and some other energy supply area.

The share of biofuels in the automotive fuel market is expected to grow rapidly in the next decade. In the USA, the environment protection agency renewable fuel standard version 2 (EPA-RFS2) and the Californial low-carbon fuel standard are driving the US market. The EPA-RFS2 requires that 36 billion gallons (136 billion liter) of renewable fuel should be available in the US market by 2022 [3]. For the transport sector of the European Union, 10% transportation fuel from biofuel is targeted by 2020 [4]. In addition, biofuels also have great potential in some other countries, such as Brazil, China, etc. in the next 10 years [5,6].

Table 1
Fuel shares of world total primary energy supply [2].

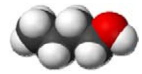
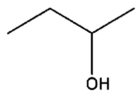
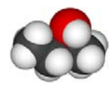
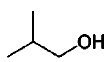
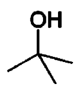
Source of energy Energy	Share
Coal	26.5%
Oil	34%
Natural gas	20.9%
Nuclear	5.9%
Hydropower	2.2%
Combustible renewables and waste	9.8%
Others*	0.7%
Total	100%

* Other includes geothermal, solar, wind, heat, etc.

For the transportation vehicles, various biofuels have been researched or applied, such as biodiesel, bioDME, biomethanol, bioethanol, biobutanol, etc. All these biofuels can be derived from renewable feedstock as opposed to from fossil feedstock in the case of gasoline or diesel fuels. One widely used biofuel is biodiesel, which is defined as the mono-alkyl esters of long chain fatty acids derived from renewable feedstock, such as vegetable oil, animal fats, algae, etc. Biodiesel, considered as a possible substitute of conventional diesel fuel, usually consists of fatty acid methyl/ethyl esters, obtained from triglycerides by transesterification with methanol/ethanol respectively [7–10]. Biodiesel has many similar properties like the diesel fuel and it can blend with convention diesel in any proportion. Many researchers like Qin et al. [11,12], Huang et al. [13], Fang and Lee [14,15], Qi et al. [16], Bhale et al. [17] have reported that power output of biodiesel was almost identical to that of diesel, and the soot, carbon monoxide (CO), carbon dioxide (CO_2) and total hydrocarbon (THC) emissions are reduced in biodiesel and its blends, because of its oxygen content which leads to more complete combustion. However, the NO_x emission is reported to be in the range between ±10% as compared to diesel depending on engines combustion characteristics [8]. In addition, biodiesel also has many other advantages in comparison to diesel fuel, such as non-toxic, easy degradation, more safe due to higher flash point, more clean combustion, 90% reduction in cancer risks, lower polycyclic aromatic hydrocarbon (PAH) and nitro PAH compounds emissions. However, there are still some disadvantages, such as difficulty in storage due to an easier degeneration, the decline of flow characteristics at low fuel temperatures, and more expensive due to less production.

The other widely used biofuels are bioalcohols. Alcohols, mainly ethanol and to a much lesser extent methanol, which are considered as alternative fuels for internal combustion engines [18–23]. Ethanol is a biomass-based renewable fuel that can be produced by alcoholic fermentation of sugar from vegetable materials, such as corn, sugar cane, sugar beets, barley, sweet sorghum, and agricultural residues [23–25], but methanol is mainly produced from coal or petrol based fuels. Therefore, ethanol is superior to methanol due to its renewability and is widely used as an additive or alternative fuel in many countries, such as the United States, Brazil, China, etc. However, there are also several critical issues that need to be considered with the use of ethanol as an engine fuel [25–28]. Ethanol is corrosive to the existing pipelines through general corrosion, dry corrosion and wet corrosion. General corrosion is caused by ionic impurities, mainly chloride ions and acetic acid. Dry corrosion is attributed to the ethanol molecule and its polarity. Some metals,

Table 2
Molecular structure and main application of butanol isomers.

Butanol isomers	Molecular structure and sketch map	Main application
1-Butanol	 	Solvents – for paints, resins, dyes, etc. Plasticizers – improve a plastic material processes Chemical intermediate – for butyl esters or butyl ethers, etc. Cosmetics – including eye makeup, lipsticks, etc. Gasoline additive
2-Butanol	 	Solvent Chemical intermediate – for butanone, etc. Industrial cleaners – paint removers Perfumes or in artificial flavors
iso-Butanol	 	Solvent and additive for paint Gasoline additive Industrial cleaners – paint removers Ink ingredient
tert-Butanol	 	Solvent Denaturant for ethanol Industrial cleaners – paint removers Gasoline additive for octane booster and oxygenate Intermediate for MTBE, ETBE, TBHP, etc.

such as magnesium, lead and aluminum are susceptible to chemical attack by dry ethanol. Wet corrosion is caused by the ethanol to absorb moisture from the atmosphere, which oxidizes most metals. And it may tend to be more corrosive as it passes through the fuel injection system. Further, non-metallic components have also been affected by ethanol with particular reference to elastomeric components. Detailed effects of corrosiveness have been reviewed by Hansen et al. [23]. Ethanol has much lower flash point than the diesel fuel and has higher vapor formation potential in confined spaces, thus requiring extra caution in its usage. Some surfactants or cosolvents must be used in order to ensure solubility of ethanol and diesel fuel.

A very competitive biofuel for use in engines is butanol. Like ethanol, butanol is a biomass-based renewable fuel that can be produced by alcoholic fermentation of the biomass feedstocks [25,29–31]. Butanol has a 4-carbon structure and is a more complex alcohol than methanol and ethanol that only has 1 and 2-carbon structure respectively. Butanol, like ethanol, can blend with gasoline very well. Furthermore, butanol could be a future option for blending with diesel. Butanol contains more oxygen content compared with the biodiesel, leading to further reduction of soot. NO_x emissions can also be reduced due to its higher heat of evaporation, which results in a lower combustion temperature [32]. Therefore, the butanol has more advantages than the widely used ethanol and biodiesel. However, the main disadvantage of butanol is its quite low production. Compared butanol yield by acetone butanol ethanol (ABE) fermentation to that of the yeast ethanol fermentation process, the yeast process yields of ethanol has a 10–30 times higher production rate. This becomes very clear why ethanol was chosen as an alternative fuel source over butanol during the oil crisis in the 1970s and 1980s. However, with the development of butanol fermentative process, a higher butanol production rate has become possible, explaining the increasing studies on it in recent years.

In this review, the properties of butanol are compared with those of the conventional gasoline, diesel fuel, and some widely used biofuels, i.e. methanol, ethanol, biodiesel. Then, the development of butanol production is reviewed and various methods for increasing the production of butanol are introduced in detailed. Next, the applications of butanol as a biofuel are summarized from three aspects. First, the fundamental combustion experiments in some burning reactors are reviewed, including the progress in the chemical kinetics. Second, the applications of butanol in SI engines, including the studies on cooperative fuel research engines. Third, the applications of butanol in CI engines, including the studies on properties of butanol–diesel blends and some new combustion technologies, i.e. homogeneous charge compression ignition (HCCI), low temperature combustion (LTC). Finally, some challenges and future research directions are outlined in this paper.

2. Properties of butanol and butanol isomers

2.1. Properties of butanol isomers

Alcohols are defined by the presence of a hydroxyl group (–OH) attached to one of the carbon atoms. Butanol has a 4-carbon structure and the carbon atoms can form either a straight-chain or a branched structure, resulting in different properties. There exist different isomers, based on the location of the –OH and carbon chain structure. The molecular structure and the main applications of butanol isomers are listed in Table 2. 1-butanol, also better known as n-butanol, has a straight-chain structure with the –OH at the terminal carbon. 2-butanol, also known as sec-butanol, is also a straight-chain alcohol but with the OH group at an internal carbon. Iso-butanol is a branched isomer with the OH group at the terminal carbon and tert-butanol refers to the branched isomer with the OH group at an internal carbon. The different structures of butanol isomers have a direct impact on the physical properties, which are summarized in Table 3. Although the properties of butanol isomers are different in octane number, boiling point, viscosity, etc., the main applications are similar in some aspects, such as being used as solvents, industrial cleaners, or gasoline additives. All these butanol isomers can be produced from fossil fuels by different methods. But

Table 3
Comparison of butanol isomers [33].

	1-butanol	2-butanol	Tert-butanol	Iso-butanol
Density (kg/m^3)	809.8	806.3	788.7	801.8
Research octane number	96	101	105	113
Motor octane number	78	32	89	94
Boiling temperature (°C)	117.7	99.5	82.4	108
Enthalpy of vaporization (kJ/kg) at T_{boil}	582	551	527	566
Self-ignition temperature (°C)	343	406.1	477.8	415.6
Flammability limits vol.%	1.4–11.2	1.7–9.8	2.4–8	1.2–10.9
Viscosity (mPa s) at 25 °C	2.544	3.096	–	4.312

butanol produced from biomass usually assumes a straight-chain molecule structure, n-butanol, which will be reviewed in this paper.

2.2. Advantageous and disadvantageous properties of n-butanol

The physical and chemical properties of gasoline, diesel fuels, methanol, ethanol, n-butanol are listed in Table 4. These properties indicate that n-butanol has the potential to overcome the drawbacks brought by the low-carbon alcohols.

(1) Higher heating value. Typically the low heating value of alcohol rises with increased carbon atom number. n-Butanol is a four carbon alcohol, doubling the carbon of ethanol and containing 25% more energy. Therefore, the fuel consumption will reduce and a better mileage can be obtained.
(2) Lower volatility. The volatility (saturation pressure) of alcohols decreases with the increase of carbon atom number. This means that n-butanol will have less tendency towards cavitation and vapor lock problem, which can eliminate the need for very special blends during the summer and winter months as the gasoline.
(3) Less ignition problems. As the heat of vaporization of butanol is less than half of that of ethanol, an engine running on butanol should be easier to start in cold weather than one running on ethanol or methanol. In addition, the autoignition temperature of the n-butanol is lower than ethanol or methanol, which also results in less ignition problems at cold start or low load conditions.
(4) Intersolubility. Alcohol molecules contain alkyl and hydroxyl, the more carbon an alcohol molecule contains, the easier the alcohol can be blended into diesel fuel. n-Butanol has very good intersolubility with diesel without any cosolvents and it can also blend with gasoline very well.
(5) Higher viscosity. The viscosity of alcohols increases with longer carbon chains. For this reason, butanol is used as an alternative to shorter alcohols when a more viscous solvent is desired. The kinematic viscosity of butanol is several times higher than that of gasoline and is about the same level as that of diesel fuel, so it will not cause potential wear problems in sensitive fuel pump designs in diesel engines due to insufficient lubricity.
(6) More safer. As butanol has a very low vapor pressure point and a high flash point, it is a much safer fuel to use in high temperatures.
(7) Easier distribution. Butanol better tolerates water contamination and is less corrosive than ethanol therefore it is more suitable for distribution through existing pipelines, whereas ethanol must be transported via rail, barge or truck. In blends with diesel or gasoline, butanol is less likely to separate from the base fuel than ethanol if the fuel is contaminated with water. This facilitates storage and distribution of blended fuels.
(8) A longer term benefit for hydrogen. Reformed butanol has four more hydrogen atoms than ethanol, resulting in a higher energy output (10 watt h/g versus 8 for ethanol) when it is used in the fuel cell. In addition, hydrogen generated during the butanol fermentation process is easily recovered, increasing the energy yield of a bushel of corn by an additional 18% over the energy yield of ethanol produced from the same quantity of corn.

n-Butanol can be produced from biomass (as "biobutanol") as well as from fossil fuels (as "petro-butanol"), but biobutanol and petro-butanol have the same chemical properties. For biobutanol, it has a number of synergies with bioethanol as following [23,36,37]:

- Biobutanol is produced from the same agricultural feedstocks as ethanol (i.e. corn, wheat, sugar beet, sorghum, cassava and sugarcane).
- Existing ethanol capacity can be cost-effectively retrofitted to biobutanol production (minor changes in fermentation and distillation).
- There is a vapor pressure co-blend synergy with biobutanol and gasoline containing ethanol, which facilitates ethanol blending.
- Offers biomass producers and the biofuel converters the option of upgrading to a higher value bio-molecule. It is also compatible with and facilitates the introduction of ethanol into the fuel pool.

Table 4
Specification of alcohols and conventional fossil fuels [34,35].

	Gasoline	Diesel	Methanol	Ethanol	n-Butanol
Molecular formula	C_4–C_{12}	C_{12}–C_{25}	CH_3OH	C_2H_5OH	C_4H_9OH
Cetane number	0–10	40–55	3	8	25
Octane number	80–99	20–30	111	108	96
Oxygen content (% weight)	–	–	50	34.8	21.6
Density (g/mL) at 20 °C	0.72–0.78	0.82–0.86	0.796	0.790	0.808
Autoignition temperature (°C)	∼300	∼210	470	434	385
Flash point (°C) at closed cup	−45 to −38	65–88	12	8	35
Lower heating value (MJ/kg)	42.7	42.5	19.9	26.8	33.1
Boiling point (°C)	25–215	180–370	64.5	78.4	117.7
Stoichiometric ratio	14.7	14.3	6.49	9.02	11.21
Latent heating (kJ/kg) at 25 °C	380–500	270	1109	904	582
Flammability limits (%vol.)	0.6–8	1.5–7.6	6.0–36.5	4.3–19	1.4–11.2
Saturation pressure (kPa) at 38 °C	31.01	1.86	31.69	13.8	2.27
Viscosity (mm^2/s) at 40 °C	0.4–0.8 (20 °C)	1.9–4.1	0.59	1.08	2.63

- Like ethanol, butanol will also assist in the conversion of vegetable oils into biodiesel.

Although the n-butanol has more benefits than the low-carbon alcohols as an engine fuel, there are still some potential issues with the direct use of butanol in the engine. These potential issues are shown as follows:

- To match the engine performance, the utilization of butanol fuel as a substitute for gasoline or diesel engines requires fuel-flow increases. Although butanol has a higher energy than ethanol or methanol, its heating value is still lower than the conventional gasoline or diesel fuel.
- Alcohol-based fuels are not compatible with some fuel system components, and alcohol fuels may cause erroneous gas gauge readings in vehicles with capacitance fuel level gauging.
- While ethanol and methanol have lower energy densities than butanol, their higher octane number allows for greater compression ratio and efficiency. Higher combustion engine efficiency allows for less greenhouse gas emissions per unit motive energy extracted.
- n-Butanol has a higher viscosity than ethanol and methanol, which will not result in lubricity and potential wear problems in sensitive fuel pump designs in diesel engines. However, a higher viscosity will incur a potential aggradation or corrosiveness problem when using the blends of gasoline and n-butanol in SI engines.

3. Progress in the production of n-butanol

3.1. The history of n-butanol production

The discovery of n-butanol as a regular constituent of fusel oil was achieved by Wirtz in 1852. Ten years later, 1862, Pasteur, from his experiments, concluded that butyl alcohol was a direct product of anaerobic conversion of lactic acid and calcium lactate. Between 1876 and 1910, several scholars researched the production of acetone–butanol and related solvents [38]. Production of industrial butanol and acetone, via ABE (acetone, butanol, ethanol) fermentation started in 1912–1916 [39,40], which is one of the oldest known industrial fermentations. It was ranked second only to ethanol fermentation by yeast in its scale of production, and is one of the largest biotechnological processes ever known. Fig. 1 shows a scheme of the respective production pathway through ABE [41]. There are three major classes of products during fermentation: (i) solvents (acetone, ethanol and butanol); (ii) organic acids (acetic acid, lactic acid and butyric acid); (iii) gases (carbon dioxide, and hydrogen). The biosyntheses of acetone, butanol and ethanol share the same metabolic pathway from glucose to acetyl coenzyme A (acetyl-CoA) but branches into different pathways thereafter. All n-butanol is biobutanol due to the fermentations production methods.

Since the 1950s ABE fermentation declined continuously, and almost all butanol was produced via petrochemical routes, so it is called petro-butanol. The production of butanol by fermentation declined mainly because the price of petrochemicals dropped below that of starch and sugar substrates such as corn and molasses. Therefore, butanol production from fossil fuel became more popular and sealed the fate of ABE fermentation during this stage. Fig. 2 shows the chemical pathway for the n-butanol production, and the original material is propylene [42].

In the 1970s, the oil crisis gave a rise to the development of biofuels. At that time, the primary focus for alternative fuels was on ethanol. People were familiar with its production and did not realize that dehydration (a very energy consuming step) was necessary

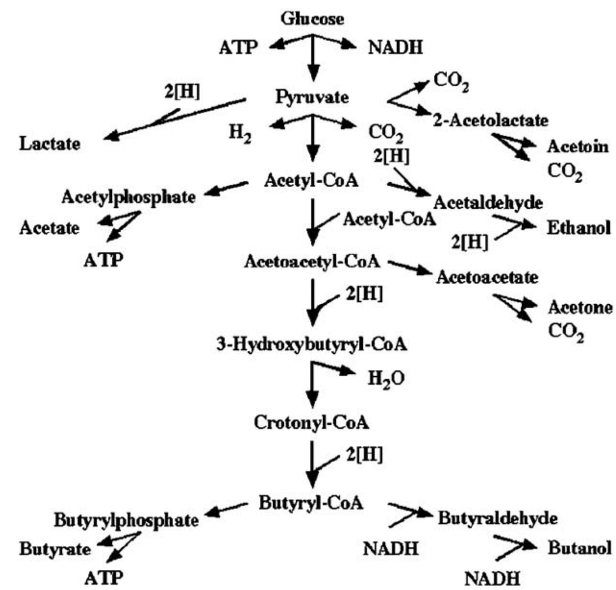


Fig. 1. Fermentation pathway of the butanol employed by *C. acetobutylicum* [41].

in order to blend ethanol with fossil fuels. Nor did we realize the difficulty of distribution of ethanol since ethanol cannot be transferred through the existing pipeline infrastructures in any practical concentration without corrosion and damage to rubber seals. Although ethanol is a lower power grade alcohol with corrosive, hard to purify, very evaporative, and dangerously explosive characteristics, a higher production of ethanol leads to its primary application as a biofuel. The ABE process uses bacteria to produce a 6:3:1 ratio of butanol, acetone and ethanol. That is, for each bushel of corn you would garner 1.3 gallons of butanol, 0.7 gallons of acetone and 0.13 gallons of ethanol with concentrations of 1–2%. However, if compare ABE yield to that of the yeast ethanol fermentation process, the yeast process yields 2.5 gallons of ethanol from a bushel of corn, with concentrations of 10–15%. As a result it becomes very clear why ethanol was chosen as an alternative fuel source over butanol in the 1970s and 1980s.

For the past 30 years the energy-intensive ethanol process still has not solved our fuel, power or clean air requirements. Currently in response to the rising cost of petrochemicals and pollution, industries are reexamining fermentation as a source of butanol in many countries. In addition, any front-end (material handling)

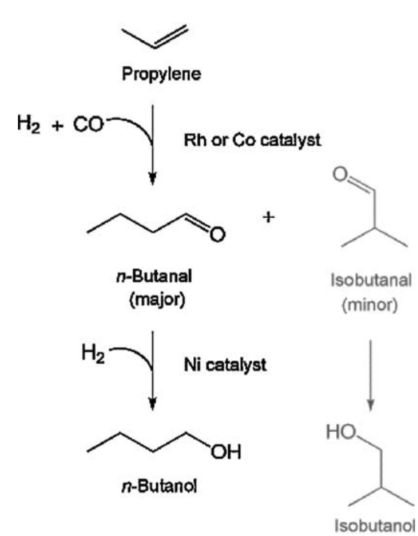


Fig. 2. Chemical pathway for n-buatnol production [42].

Table 5
Recent patens of microbial strains and fermentation processes for butanol production.

Patent no.	Patent title	Butanol titer	Main inventor and the year of publication
US4777135	Methods for producing butanol by fermentation	22.8 g/L	Husted GR, 1988
US20026358717	Methods of producing butanol using a mutant strain of *C. beijerickil*	20.6 g/L	Blaschek H, 2002
US20050089979	Process for continuous solvent production	251.3 g/L	Ezeji TC, 2005
US20070020740	Blocking sporulation by inhibiting SPOIIE	153 mmol/L	Bennett GN, 2007
WO2007041269	Fermentative production of four carbon alcohols	0.01–0.8 mmol/L	Donaldson GK, 2007
WO2008052991	Butanol production in a eukaryotic cell	0.015–0.02 g/L	Madeleine RL, 2008
US20080182308	Fermentative production of four carbon alcohols	0.01–1.03 mM	Donaldson GK, 2008
WO2008072920	Method for preparing butanol through butylryl-CoA as an intermediate using bacteria	0.018 g/L	Papoutsakis ET, 2008
US20090162911	Strain for butanol production	0.04 mmol/L	Larossa RA, 2009
US20100205857	Butanol production in a eukaryotic cell	30 g/L	Dijk E, 2010
WO2010000649	Method for the combined production of butanol and hydrogen	160 mmol/L	Classen P, 2010

technology that is applicable to present sugars to an ethanol facility can be used by a butanol biorefinery with a small modification to the fermentation parlor. As a result many countries or big oil company pay more attention to the n-butanol again.

3.2. Current butanol productions in the world

3.2.1. Current butanol productions situation

As the largest players in the area of advanced biofuels, BP and DuPont announced their partnership to develop and commercialize biobutanol in 2006, and presented plans to produce 30,000 t butanol per year in a modified ethanol facility of British Sugar in the UK. Test results from BP and DuPont in 2008 showed that the use of biobutanol can increase the blending of biofuels in gasoline beyond the current 10% limit for ethanol without compromising performance [43]. There are a number of biotechnology companies, such as Butyl Fuel, Cathay Industrial Biotech, Cobalt Biofuels, Green Biologics, Metabolic Explorer, Tetravitae Bioscience, and others around the world dedicated to providing strains and process solutions for ABE fermentation for industrial customers. Various inventions also have been reported for the biological production of butanol, maintaining its competitiveness in efficiency, economy, and production scale. Some patented inventions and relevant research achievements about the microbial strains and fermentation processes for the enhanced production of biobutanol were reviewed in Table 5. Other patents review on the butanol production can be found in Kharkwal et al. [44].

3.2.2. General aspects of biobutanol production

3.2.2.1. Microorganisms. Butanol (acetone, ethanol, and isopropanol) are naturally formed by a number of clostridia. In addition, clostridia are rod-shaped, spore-forming Gram positive bacteria and typically strict anaerobes [45]. A typical feature of the clostridial solvent production is biphasic fermentation. The first phase is the acidogenic phase, during which the acids forming pathways are activated, and acetate, butyrate, hydrogen, and carbondioxide are produced as major products. This acidogenic phase usually occurs during the exponential growth phase. The second phase is the solventogenic phase during which acids are reassimilated and used in the production of acetone, butanol and ethanol (or isopropanol instead of acetone in some *Clostridium beijerinckii* strains). The transition from acidogenic to solventogenic phase is the result of a dramatic change in gene expression pattern.

Solventogenesis closely coupled to sporulation [46]. The transcription factor responsible for initiation of sporulation (Spo0A) also initiates solvent production. Spo0A deletion mutants are severely deficient in solvent production and fails to septate, while strains with amplified Spo0A overexpress solventogenic genes but fail to produce more solvent due to an accelerated sporulation process [47]. Thus selective utilization of positive effect of Spo0A on solvent formation is required for enhanced solvent production. For this purpose, it is recommended that Spo0A should be evolved to a solventogenic gene activating regulator without the sporulation activating function [48].

3.2.2.2. Fermentation. Recent advances in butanol fermentation including upstream and downstream processing were evaluated by Ezeji et al. [49]. The use of excess carbon under nitrogen limitation is required to achieve high levels of solvent production. Iron is an important mineral supplement since the conversion of pyruvate to acetyl-CoA involves a ferredoxin oxidoreductase iron-sulfur protein. The pH of the medium is very important to the biphasic acetone–butanol fermentation [50].

Economic analysis demonstrated that the fermentation substrate was one of the most important factors that influenced the price of butanol [51]. Since the cost of the fermentation substrate has the greatest influence on the price of butanol, some renewable and economically feasible substrates were investigated in the butanol fermentation production. The substrates considered for acetone–butanol production includes starch-based packaging materials [52], corn fiber hydrolysate [53], soy molasses [54], fruit processing industry waste [52], and whey permeate [55]. The total solvents produced from these alternative renewable resources ranged from 14.8 to 30.1 g/L [49].

3.2.2.3. Solvent toxicity. One of the most critical problems in ABE fermentation is solvent toxicity. Clostridial cellular metabolism ceases in the presence of 20 g/L or more solvents [56]. This limits the concentration of carbon substrate that can be used for fermentation resulting in low final solvent concentration and productivity. The lipophilic solvent butanol is more toxic than others as it disrupts the phospholipid components of the cell membrane causing an increase in membrane fluidity [57]. Also, butanol is the only solvent produced to the level that becomes toxic to the cells during the fermentation of clostridia [58].

3.3. Improvements in butanol production

Three major factors determine the economic competitiveness of the biotechnological butanol production: substrate costs, low product yields versus solvent toxicity, and costs for downstream processing. The valuable improvements of butanol production will be reviewed from these aspects: metabolic engineering of *Clostridium acetobutylicum*, approaches for improving fermentation process and butanol recovery technology, economic substrates application for butanol fermentation.

3.3.1. Metabolic engineering of C. acetobutylicum

The objectives of metabolic engineering for butanol producing clostridia include enhancing butanol production with respect to

Table 6
Fermentation performance of metabolically engineering *C. acetobutylicum* strains.

Up-regulated	Down-regulated	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)	Total solvent	B%	Comment	References
Control		4.5	9.5	0.74	14.7	65%	50% higher yield (g/L) of solvents	[73]
Adc, *ctfAB*	–	8.7	13	1.4	23.1	56%	On glucose	
Control		3.8	5.5	0.7	16.4	34%	*B%* is increased	[63]
adhE	–	4.2	8.7	1.9	14.8	59%		
–	pSOL1	0	0	0	0	N/AB	*AdhE* is responsible for ABE	[74]
adhE	pSOL1	0	6.2	0.4	6.6	93%	production	
Control		4.6	9.7	0.5	14.8	66%	Butyryl-P level is critical for butanol	[75]
–	*pta*	4.2	9.9	0.6	14.7	67%	production	
–	*buk*	2.3	10.8	0.7	13.8	78%		
Control		3.8	5.5	0.7	10.0	55%	Improed yields of solvent	[65,76]
–	*solR*	8.1	17.8	1.0	26.9	66%	High butanol concentration achieved	
–	*buk*	4.4	16.7	2.6	23.7	70%		[77]
adhE	*buk*	3.8	16.7	4.5	25	67%		
Control		5	11.7	0.73	17.4	67%	Controlling regulator of solvent	[78]
–	*solR*	5.6	14.6	4.4	24.6	59%	producing genes	
adhE	*solR*	8.2	17.6	2.1	27.9	63%	High butanol production achieved	
Control		4.4	10.4	0.2	15	69%	Highest ethanol production/as RNA	[79]
adhE	–	1.4	10	8.8	20.2	50%	regulation	
–	*ctfB*	0.3	1.2	0.2	1.7	71%		
Wild-type		3	11	1.1	15.1	73%	Solvent tolerate strain	[80]
Control		6	13	1.1	20.1	65%		
groESL	–	8	17	1.1	26.1	65%		
Control		2.83	13.6	2.7	19.13	71%	Increase the butanol ratio of	[81]
–	*adc*	0.21	7.4	1.66	9.27	80%	*C.acetobutylicum* by *adc* disruption	

final concentration and productivity, increasing butanol (solvent) tolerance, extending substrate utilization range, increasing butanol yield on carbon source, and selective production of butanol instead of mixed acids/solvents production.

The first genes encoding solventogenic enzymes from *C. acetobutylicum* were cloned and sequenced in 1990 [59–61]. Meanwhile, the complete genome sequence has been determined. Several examples of metabolic engineering of *C. acetobutylicum* have been reported over the years.

The first successful metabolic engineering example was the amplification of the acetone formation pathway in *C. acetobutylicum*. As shown in Fig. 3, the acetone producing pathway is coupled with that leading to the formation of the precursor of butanol, butyryl-CoA, from butyrate. In the fermentation of recombinant *C. acetobutylicum* with amplified *adc* (encoding acetoacetate decarboxylase) and *ctfAB* (encoding CoA transferase) genes, the acetone-forming enzymes became active earlier, which led to earlier induction of acetone formation. This resulted in increased final

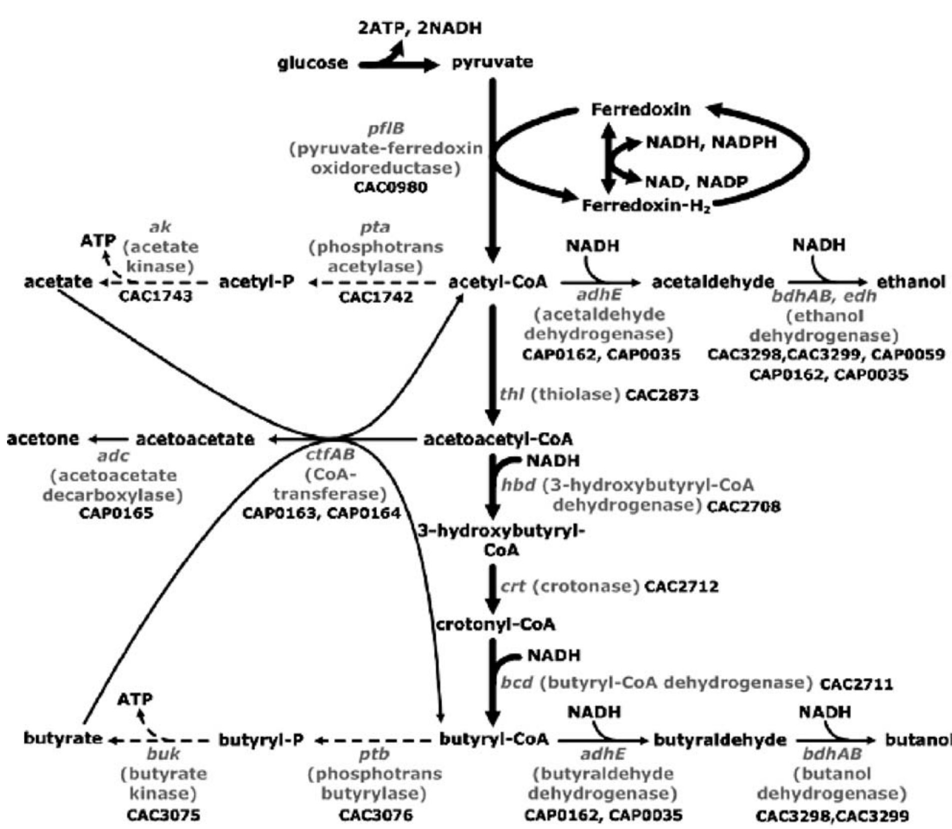


Fig. 3. Metabolic pathways in *C. acetobutylicum* [48].

concentrations of acetone, butanol, and ethanol by 95%, 37%, and 90%, respectively, compared to its parental strain [62].

The strain lacking pSOL1 but expressing the *AdhE* gene was found to produce butanol and ethanol [63]. As shown in Fig. 3, *AdhE* is responsible for the formation of both acetaldehyde and butyraldehyde. However, the effect of its overexpression on solvent formation appears to depend on the physiological state of the cell. When the control strain produces less than 10 g/L of butanol, the *adhE* gene overexpression results in increased butanol production. On the other hand, when the control strain produces more than 10 g/L of butanol, overexpression of the *adhE* gene leads to production of more ethanol than butanol.

Further studies were carried out to understand the regulation involved in solvent formation by characterizing the fermentation performance of the *solR* (encoding the regulator of solvent production genes) mutant strain [64]. SolR was suggested to be a putative DNA binding transcriptional repressor that negatively regulates solventogenic metabolism [65]. Thus, SolR is induced in acidogenesis and suppressed in solventogenesis. In the solR inactivated strain, the expression of the solventogenic genes is induced earlier and maintained throughout the solventogenic phase. However, Thormann et al. [66] reported that the aforementioned result was incorrect because the DNA clone used to complement the phenotype of the *solR* knock-out also contained the binding site of a regulator, possibly Spo0A or another regulator, which affected the sol operon expression by a regulator titration mechanism [67]. They suggested that Spo0A, rather than SolR, is a putative regulator of the sol operon. Further studies are needed to clarify the detailed control mechanism of solventogenesis. Nonetheless, removing the *solR* gene region (which contains the putative Spo0A binding motif) allowed enhanced butanol production.

Desai and Papoutsakis [68] examined the effectiveness of antisense RNA (asRNA) in reducing the activities of butyrate forming enzymes in *C. acetobutylicum*. They designed *buk* and *ptb* two different asRNAs, and the result showed the potential of asRNA for the strain improvement through the fine-tuning of gene expression. While the strain transformed with the *buk* asRNA produced 50% and 35% higher final concentrations of acetone and butanol, respectively, the strain transformed with the ptb-asRNA exhibited 96% and 75% lower final acetone and butanol concentrations, respectively. The results obtained with the strain transformed with the buk-asRNA were similar to those obtained with the buk-inactivated strain (PJC4BK), which showed 65–75% lower butyrate-formation flux yet produced more solvents than the wild-type strain, as mentioned above. Continued work with the asRNA approach revealed that CoA transferase, rather than acetoacetatede carboxylase, is the rate-limiting enzyme in the acetone formation pathway [69].

The solvent tolerance and stress response of *C. acetobutylicum* involves the overexpression of many genes including molecular pumps, chaperones (e.g., *groES*, *dnaKJ*, *hsp18*, and *hsp90*), and genes involved in sporulation, fatty acid synthesis and transcriptional regulators [70]. Genomic library screening has been used to identify individual genes capable of improving solvent tolerance [71]. In general, targeting transcriptional regulators may be the most efficient strategy to realize complex phenotypes that requires intensive reprogramming of the cellular metabolism. As illustrated by Alper et al. [72] for ethanol tolerance in yeast, this may be realized by altering global transcription regulators. Finally, some important research results were reviewed in Table 6.

3.3.2. Approaches for improving fermentation process

3.3.2.1. Advanced fermentation technologies. Initial studies employing *C. beijerinckii* BA101 were performed in batch reactors ranging in size from 1 to 10 L using cracked corn, cornstarch, maltodextrin, and glucose. Under batch operation conditions, the culture was able to produce 18–33 g/L ABE in 72 h of fermentation using glucose or starch as the substrate, the productivity was 0.25–0.46 g/L/h. Productivity in batch reactors is often low due to downtime, long lag phase, and product inhibition. This problem can be eliminated using fed-batch techniques or continuous culture with the application of novel product removal techniques [49].

Another reason for low reactor productivity is the low concentration of cells in the bioreactor. In a batch reactor, cell concentration over 3 g/L is rarely achieved. Therefore, reactor productivity can be improved by increasing the cell concentration in the reactor. An increased cell concentration can be achieved by fixing cells onto supports or gel particles. Another option can be choosed is the application of a membrane that returns cells to the reactor while the aqueous solution containing the product permeates the membrane. The following sections describe the fed-batch and continuous fermentation for butanol production.

3.3.2.1.1. Fed-batch and continuous fermentation. Fed-batch fermentation is an industrial technique applied to processes where a high substrate concentration is toxic to the culture. In such cases, the reactor is initiated in a batch mode with a low substrate concentration (non inhibitory to the culture) and a low medium volume. As the substrate is used by the culture, it is replaced by adding a concentrated substrate solution at a slow rate thereby keeping the substrate concentration in the fermentor below the toxic level for the culture. The culture is harvested when the liquid volume is approximately 75% of the volume of the reactor. Since butanol is toxic to *C. acetobutylicum* or *C. beijerinckii* cells, the fed-batch fermentation technique cannot be applied unless one of the novel product recovery techniques is applied for simultaneous separation of product. This process was employed for butanol fermentation using *C. beijerinckii* where a glucose solution containing 500 g/L glucose was fed and product was recovered by gas stripping or pervaporation, which has been reviewed by Ezeji et al. [82].

The continuous culture technique can be used to improve reactor productivity and to study the physiology of the culture in a steady state. In such systems, the reactor is initiated in a batch mode and cell growth is allowed until the cells are in the exponential phase. While the cells are in the exponential phase, the reactor is fed continuously with the medium and a product stream is withdrawn at the same flow rate as the feed, thus keeping a constant volume in the reactor. This technique of butanol production by *C. beijerinckii* BA101 was investigated by Formanek et al., and resulted in a productivity rate of 1.74 g/L/h [83]. In a single-stage continuous system, high reactor productivity may be obtained, but this occurs at the expense of low product concentration when compared to that achieved in a batch process. Because of the fluctuating solvent level and the complexity of butanol fermentation, the use of a single-stage continuous reactor does not yet seem practical on an industrial scale. Two or more multistage continuous fermentation systems have been investigated to reduce fluctuation and increase solvent concentration in the product stream [84].

3.3.2.1.2. Immobilized cell continuous reactors. High cell concentrations result in high reactor productivity. Such systems are continuous where feed is introduced into a tubular reactor at the bottom with product escaping at the top. These systems are often non-mixing reactors where product inhibition is significantly reduced. To improve the reactor productivity, *C. beijerinckii* cells were immobilized onto clay brick particles by adsorption and achieved reactor productivity on the order of 15.8 g/L/h [85]. In these scaled-up reactors, productivity 40–50 times greater than that obtained in batch reactors was achieved. This result suggests that immobilized cell continuous reactors could be used to achieve higher reactor productivity which leads to economic advantage.

3.3.2.1.3. Membrane cell recycle reactors. Membrane cell recycle reactors are another option for improving reactor productivity. In such systems, the reactor is initiated in a batch mode and cell growth is allowed. Before reaching the stationary phase, the fermentation broth is circulated through the membrane. The membrane allows the aqueous product solution to pass while retaining the cells. The reactor feed and product (permeate) removal are continuous and a constant volume is maintained in the reactor. In such cell recycle systems, cell concentrations of over 100 g/L can be achieved, and reactor productivity on the order of 6.5 g/L/h has been achieved in this system. Although superior membranes have been developed, fouling of the membrane with the fermentation broth remains a major obstacle [49].

3.3.2.2. Butanol recovery technology. High product recovery cost is another problem in biological butanol production. Besides the traditional distillation process, several other processes including pervaporation, adsorption, liquid–liquid extraction, gas stripping, and reverse osmosis have been developed to improve recovery performance and reduce costs [82]. The traditional recovery process employing distillation suffers from a high operation cost due to the low concentration of butanol in the fermentation broth. To solve this problem and the solvent toxicity problem at the same time, in situ recovery systems have been employed. From an economic point of view, reverse osmosis is most preferable. However, it has disadvantages of membrane clogging or fouling. In contrast, liquid–liquid extraction has high capacity and selectivity, although it can be expensive toper form. Thus, there are advantages and disadvantages of using each recovery system, which need to be thoroughly examined.

3.3.2.2.1. Gas stripping. Gas stripping is a simple technique for recovering butanol (acetone or ethanol) from the fermentation broth, as illustrated in Fig. 4(a) [86]. Either nitrogen or the fermentation gases (CO_2 and H_2) are bubbled through the fermentation broth followed by passing the gas (or gases) through a condenser. As the gas is bubbled through the fermentor, it captures the solvents (butanol or ABE). The solvents then condense in the condenser and are collected in a receiver. Once the solvents are condensed, the gas is recycled back to the fermentor to capture more solvents. This process continues until all the sugar in the fermentor is utilized by the culture. Gas stripping has been applied to a batch reactor to recover solvents from the fermentation broth of *C. beijerinckii* BA101 [87]. A 161.7 g/L sugar solution was successfully fermented and 75.9 g/L total solvent produced in the integrated process. Fed-batch fermentation was also integrated with gas stripping to reduce substrate inhibition and increase cell mass [88]. In this system, 500 g glucose was consumed and 233 g solvent was produced with the productivity of 1.16 g/L/h and the yield of 0.47 g/g [48].

3.3.2.2.2. Liquid–liquid extraction. Liquid–liquid extraction is another technique that can be used to remove solvents (acetone, butanol, ethanol) from the fermentation broth. In this process, an extraction solvent is mixed with the fermentation broth. Butanol, acetone, and ethanol are extracted into the extraction solvent and recovered by back extraction into another extraction solvent or by distillation. Some of the requirements for extractive butanol fermentation are summarized in Qureshi and Blaschek [88].

Common extractants employed include decanol and oleyl alcohol. Liquid–liquid extraction has critical problems, however, such as the toxicity of the extractant to the cell and emulsion formation. These problems can be overcome if the fermentation broth and the extractant are separated by a membrane that provides surface area for butanol exchange between the two immiscible phases; this is termed “Perstraction”, as illustrated in Fig. 4(b) [89]. The fermentation broth was circulated through the membrane and the bacteria were returned to the fermentor while the permeate was extracted with decanol to remove the butanol. This system achieved a productivity rate of 3.08 g/L/h. In cell recycle systems without extraction, productivity as high as 6.5 g/L [83].

3.3.2.2.3. Pervaporation. Pervaporation is a membrane-based process that allows selective removal of volatile compounds from fermentation broth, as illustrated in Fig. 4(c). The membrane is placed in contact with the fermentation broth and the volatile liquids or solvents diffuse through the membrane as a vapor which is recovered by condensation. Both liquid and solid pervaporation membranes have been used. The selectivity of this liquid membrane was better than that of a silicon rubber membrane. When pervaporation using an oleyl alcohol liquid membrane was employed for the pretreatment of butanol purification, the energy requirement was just one-tenth of that of conventional distillation. To develop a stable membrane having a high degree of selectivity, Qureshi et al. [90] synthesized a silicon-silicalite membrane which showed a 2.2-fold improvement in selectivity. Using this membrane in an integrated batch-pervaporation process with *C. beijerinckii* BA101, a 2-fold increase in the total solvent concentration (from 24.2 g/L in control batch to 51.5 g/L in batch pervaporation) was achieved. The pervaporation condition did not affect the growth of *C. beijerinckii* BA101. Since the membrane permeate contains acetone, butanol, and ethanol, distillation is still required for further purification. Pervaporation was also applied to

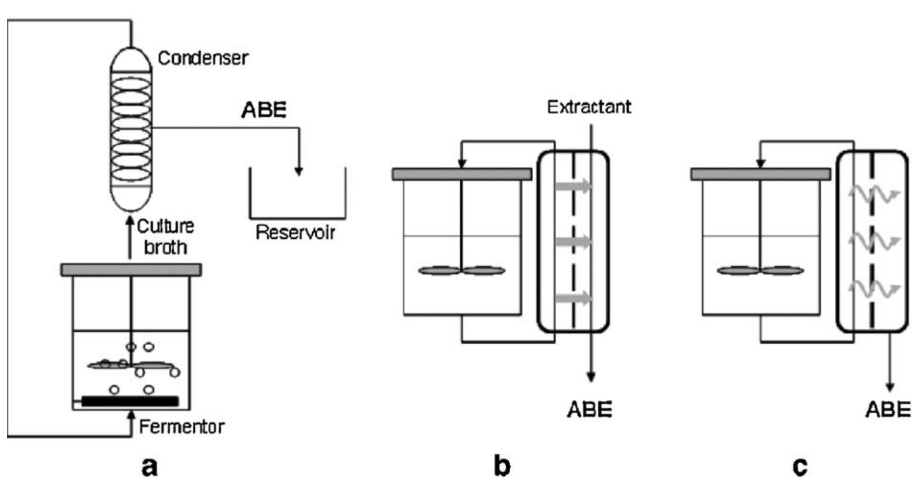


Fig. 4. Integrated systems for fermentation and in situ solvent recovery: fermentation coupled with (a) gas stripping, (b) liquid–liquid extraction (perstraction), (c) pervaporation [48].

Table 7
Production of butanol from different substrates using *C. beijerinckii*.

Fermentation parameters	Fermentation substrates								
	Glucose	Corn starch	Maltodextrins	Soy molasses	Agricultural waste	Packaging peanuts	Wheat bran	Corn fiber	DDGC
Acetone (g/L)	4.3	7.7	6.8	4.2	4.8	5.7	2.2	NC	2.7
Butanol (g/L)	19.6	15.8	18.6	18.3	9.8	15.7	8.8	NC	8.3
Ethanol (g/L)	0.3	1.2	0.7	0.3	0.2	0.3	0.8	NC	0.5
Total ABE (g/L)	24.2	24.7	26.1	22.8	14.8	21.7	11.8	20.4	11.5
ABE productivity (g/L h)	0.34	0.34	0.37	0.19	0.22	0.20	0.16	0.20	0.16
Reference	[94]	[95]	[96]	[97]	[95]	[95]	[98]	[99]	[100]

a fed-batch reactor resulting in increased solvent productivity from 0.35 (batch reactor) to 0.98 g/L/h (fed-batch reactor) due to the reduction in product inhibition [80,81]. Recently, the overall solvent productivity in continuous fermentation of *C. acetobutylicum* was increased up to 2.34 g/L/h by integrating with a pervaporation system using an ionic liquid polydimethylsiloxane ultrafiltration membrane [91].

Some other recovery techniques based on the membranes are also tested in reference [92,93]. Reverse osmosis is another recovery technique that relies on membranes. It is necessary to remove the suspended vegetative organisms using a hollow-fiber ultra filter before the reverse osmosis is carried out. After the pretreatment, reverse osmosis starts to dewater the fermentation liquor by rejecting solvents but allowing water to pass through the membrane. Consequently, the products are concentrated, and the volume of liquid to be distilled is dramatically reduced [93].

It should be emphasized that the recovery and purification processes are directly affected by the performance of fermentation, which in turn is affected by the strain characteristics. For example, when a strain is metabolically engineered to produce butanol without or much less acetone and ethanol, the purification process will be considerably simplified. When the butanol tolerance of a strain is increased by metabolic engineering, this will also facilitate the recovery process as higher butanol concentration can be achieved during the fermentation. Thus, the overall process needs to be optimized from strain development to fermentation to downstream processes. This will lead to the reduction in overall production costs.

3.3.3. Using of non-food biomass to improve the butanol production

As aforementioned, bioalcohol fuel is one of the main biofuels currently used as a petroleum-substitute in transport applications. However, conflicts over food supply and land use have made its production and utilization a controversial topic. Second generation bioalcohol production which based on bio-chemical conversion of non-food lignocellulose, offers some potential advantages over existing, energy-intensive bioethanol production processes.

A hyperamylolytic *C. beijerinckii* BA101 has an enhanced capability to utilize starch and accumulate higher concentrations of butanol (17–21 g/L) in the fermentation medium [84]. In addition to the use of corn, liquefied corn meal and corn steep liquor (a by-product of corn wet milling process that contains nutrients leached out of corn during soaking) were also tested for acetone–butanol production. In the batch process with recovery, 60 g/L of liquefied corn meal and corn steep liquor yielded ca. 26 g/L of solvent. Since the cost of the fermentation substrate has the greatest influence on the price of butanol, some other renewable and economically feasible substrates such as starch-based packaging materials, corn fiber hydrolysate, soy molasses, fruit processing industry waste, and whey permeate were investigated for butanol fermentation. The total solvents produced from these alternative renewable resources ranged from 14.8 to 30.1 g/L [49]. Among the total polysaccharides, researchers focus on celluloses and hemicelluloses which are the most abundantly renewable and available resources on the planet. A number of cabohydrates have been used for butanol production when using *C. beijerinckii* BA101 in batch fermentation, the detail data is shown in Table 7.

Wheat bran, a by-product of the wheat milling industry, consists mainly of hemicellulose, starch and protein. The wheat bran hydrolysate by dilute sulfuric acid contained 53.1 g/L total reducing sugars, including 21.3 g/L of glucose, 17.4 g/L of xylose and 10.6 g/L of arabinose [98].

A potential industrial substrate liquefied corn starch (LCS) has been employed for successful acetone butanol ethanol (ABE) production. Fermentation of LCS (60 g/L) in a batch process resulted in the production of 18.4 g/L ABE, comparable to glucose: yeast extract based medium (control experiment, 18.6 g/L ABE). A batch fermentation of LCS integrated with product recovery resulted in 92% utilization of sugars present in the feed. When ABE was recovered by gas stripping (to relieve inhibition) from the fed-batch reactor fed with saccharified liquefied cornstarch (SLCS), 81.3 g/L ABE was produced compared to 18.6 g/L (control). In this integrated system, 225.8 g/L SLCS sugar (487% of control) was consumed. A combination of fermentation of this novel substrate (LCS) to butanol together with product recovery by gas stripping may economically benefit this fermentation [101]. Unfortunately, during acid hydrolysis of the corm fiber, a complex mixture of microbial inhibitors is generated, e.g. hydroxymethyl furfural (HMF), and acetic, ferulic, glucuronic, ρ-coumaric acids, etc. However, Ezeji demonstrate in the paper that 0.3 g/L ρ-coumaric and ferulic acids were introduced into the fermentation medium, growth and ABE production by *C. beijerinckii* BA101 decreased significantly. Furfural and HMF are not inhibitory to *C. beijerinckii* BA101; rather they have stimulatory effect on the growth of the microorganism and ABE production [99].

To our knowledge, the ABE plants in Russia were the only full scale industrial plants that used hydrolyzates of lignocellosic waste for butanol fermentation. A biorefinery concept for the use of all by-products has been elaborated and was partially put into practice. The experience gained in the Russia forms a promising basis for the development of modern large scale processes to replace a considerable fraction of the current chemical production of fuel for our future needs on a sustainable basis. As the process shown in Fig. 5, during ABE production, about 50% (w/w) of the fermented sugars were converted to the gases CO_2 and H_2, and 33–39% to solvents. At the end of an average fermentation, the broth contained 6.4 g acetone, 10.0 g butanol, and 1.5 g ethanol per liter. The mass flow of the Evremovo plant in Russia can be calculated approximately as below: about 40.5 t of starch equivalents, about 90.0 t of flour dry weight, partially substituted by molasses and hydrolyzed per year were sterilized in 80.0 t of liquid substrate and fermented to 15.0 t of solvents containing 4.14 t acetone, 8.55 t butanol, 2.31 t ethanol, and to 8.7 million m^3 hydrogen gas, and 13.1 million m^3 carbon dioxide. The fermentation broth was used for the production of 11 million m^3 biogas (containing methane gas) and an unknown amount of vitamin B_{12} [102].

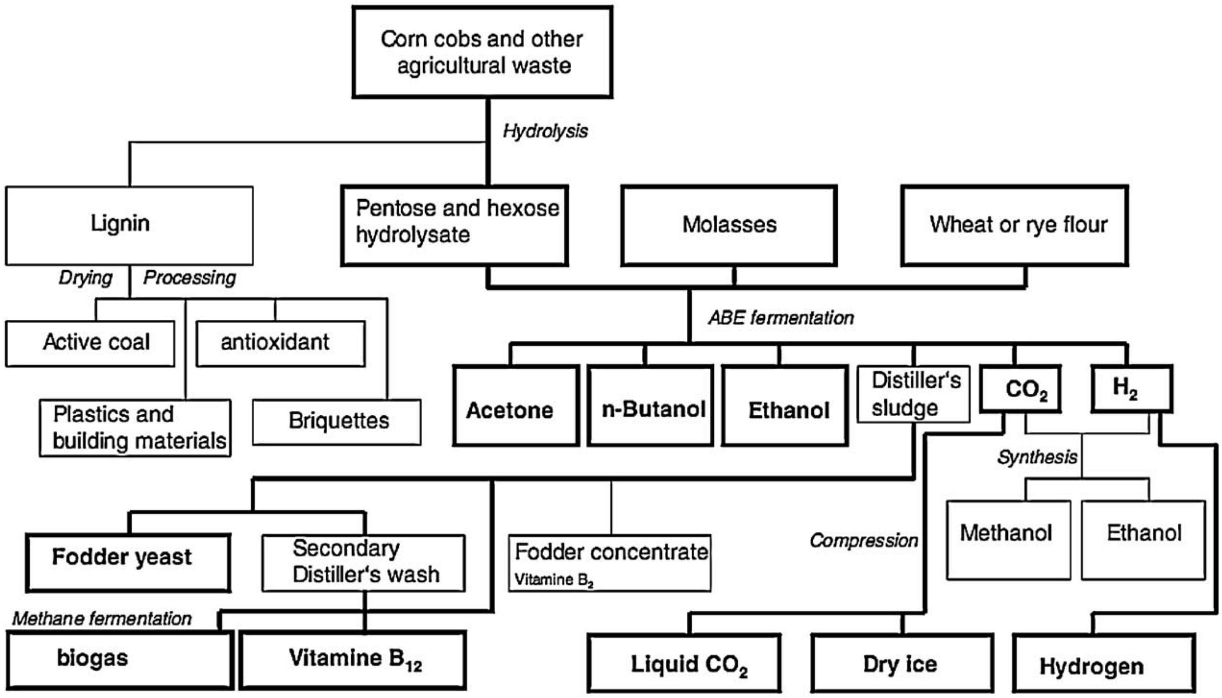


Fig. 5. The biorefinery concept uwed for AB fermentatio plants in Russia [103]. (The major substrates ans products aren shown in bold boxes. Only the processes of the biorefinery concept marked in bold print were realized.)

3.4. Efforts on Escherichia coli and Saccharomyces cerevisiae as alternative hosts for biobutanol synthesis

Though the biobutanol production by clostridia fermentation is up to 20 g/L in batch fermentation, clostridia are not ideal because of the relative lack of genetic tools to manipulate their metabolism, their slow growth, their intolerance to butanol above 1–2% and oxygen, and their production of butyrate, acetone and ethanol as by-products. Considering the rapidly expanding genomic information, molecular biology techniques, and high-throughput tools, metabolic engineers have made significant progress in constructing non-native organisms for the production of fuel-grade compounds beyond the scope of what native organisms can produce. A number of different organisms such as *S. cerevisiae*, *E. coli* and algae are being studied for biobutanol production.

Among these organisms, *E. coli* is now the most mature genetic tool as it grows quickly and genetic tools for its modification are well developed [103,104]. Over the last few years, many scientists have been involved in this work. Atsumi and Liao inserted the genes (*thl*, *hbd*, *crt*, *bcd*, *etfAB* and *adhE2*) coding the six enzymes of the butanol synthetic pathway of clostridia into *E. coli*. Under an aerobic condition, 139 mg/L of butanol was finally produced [103]. In the same way, Inui et al. inserted different combinations of the genes (*thiL*, *hbd*, *crt*, *bcd–etfB–etfA*, and *adhel* or *adhe*) into *E. coli*. Under an aerobic condition, the *E. coli* stains BUT1 with adhe1 and BUT2 with adhe were shown to produce 296 mg/L and 1184 mg/L butanol, respectively [105]. Though the titer is lower than clostridia, it still showed the feasibility to get a high butanol production using *E. coli* as a host. In addition, Atsumi et al. presented a metabolic engineering approach using *E. coli* to produce higher alcohols including butanol. This strategy uses the host's highly active amino acid biosynthetic pathway and diverts its 2-ketoacid intermediates for alcohol synthesis. Although the production is still low, the strategy described above opens up an unexplored frontier for biofuels production, both in *E. coli* and in other microorganisms [93].

As another non-native butanol producer, *S. cerevisiae* has inherent tolerance to solvents with widespread use for industrial production of ethanol, and the ability to withstand oxygen, unlike clostridia, it has the ability to withstand oxygen, therefore it might become an ideal host for industrial butanol production. In Steen's research group at the University of California, Berkeley, after related enzymes were genetic modified, the best combination achieved was a butanol titer is around 2.5 mg/L [106].

On the other aspect, an emerging rise in investment from petrochemical majors and governments for projects in Asia, Europe and the Americas aims at extracting sugars from seaweed for biobutanol. Algae-biobutanol is regarded as a promising direction for biobutanol production [107].

3.5. Summary and future directions

In this section, the production of n-butanol was overviewed. The history and current production of butanol were introduced and the methods for improving the biobutanol production were also discussed.

Butanol from biomass is a better renewable fuel than ethanol in terms of replacing gasoline in the near future. Clostridia can secrete numerous enzymes that facilitate the breakdown of polymetic carbohydrates into monomers for biobutanol production. Genetic modification is a viable method to improve the solvent production and butanol production ratio. In order to construct an engineered bacterium, the following four methods maybe applied: inserting or knocking out/down the genes coding critical enzymes, regulating some genes to enhance the strain's solvent tolerance, inserting some enzyme genes from other bacteria to improve the ability to breakdown cheaper substrate and performing genetic modification to restrict or delay the sporulation.

Compared to the strain development, the development of fermentation processes has been explored extensively, but further improvement is still need. Development of new fermentation and recovery processes based on the metabolically engineered strain will be essential for a bio-based butanol process to become economically feasible.

In addition, one of the most critical factor affecting not only butanol production but the whole bioindustry is securing low price substrates for the processes. To compete with the conventional fossil resource-based chemical industry, the biotechnology industry needs a reliable, cost-effective raw materials infrastructure. Environmental impacts and sustainability are also important issues.

In the future, much improvement is still needed to make a biobutanol process economically competitive. The next steps for improving efficiency and productivity in the production of biosolvents by *C. acetobutylicum* will probably continue depending on a two-pronged approach that involves genetics (biological strain development) and upstream and downstream manipulations (advanced fermentation technologies). Availability of genomic information for *C. acetobutylicum* will enable examination of global gene expression during the transition from acidogenesis to solventogenesis. A more complete understanding of gene expression will enable the development of improved second generation strains with improved utility for fermentation.

The ABE process using integrated gas stripping for the production of butanol has dramatically increased the efficiency and productivity of fermentation-based butanol production to the point that this process is expected to become competitive with petrochemically derived butanol. Given that additional upstream and downstream advances will continue, ABE fermentation has a bright future for the production of biosolvents from renewable resources. Continued advances in upstream and downstream processes will result in improved efficiency of bio-based solvent production. Efficient production of biosolvents will have a positive environmental impact and allow for the use of low value corn dry milling products and coproducts [cornstarch, distillers dry grain solids (DDGS)], thereby adding value to this process.

4. Progress in applications of n-butanol as a biofuel

As aforementioned, n-butanol has the potential to overcome the drawbacks brought by low-carbon alcohols, and many new methods have increased the production of n-butanol. On the other hand, some factors, such as uncertain oil price, greenhouse gas emission, and the need for increased energy security and diversity, promote the development of biofuels. Thus, investigations of n-butanol as an alternative biofuel have been conducted by several research groups recently, in which butanol has been blended with either gasoline or diesel fuel for engine application, or has been studied on some fundamental burning reactors.

4.1. Fundamental combustion experiments and chemical kinetics

In fundamental combustion experiments of n-butanol, the laminar burning velocity was measured, and the intermediate species formed in premixed combustion or diffusion combustion were studied. The chemical kinetic model of n-butanol was developed using these experiments as validation data. These predictive models can provide a better understanding of the combustion characteristics of n-butanol and explain their difference from petroleum derived materials or other biofuels.

4.1.1. Studies of fundamental combustion in some burning reactors

An early study on a static reactor reported that the pyrolysis of n-butanol is initiated by fission at the C_3H_7–CH_2OH bond to produce the n-propyl radical and hydroxymethyl radical. The hydroxymethyl radical further decomposes to formaldehyde and a hydrogen radical, while the n-propyl radical decomposes to ethylene and a methyl radical [108]. In another study, Roberts measured the burning velocities of n-butanol using shadowgraph images of the flame cone [109]. The results indicated that the maximum burning velocity of n-butanol is similar to that of n-propanol and isopentyl alcohol (approx. 46 cm/s).

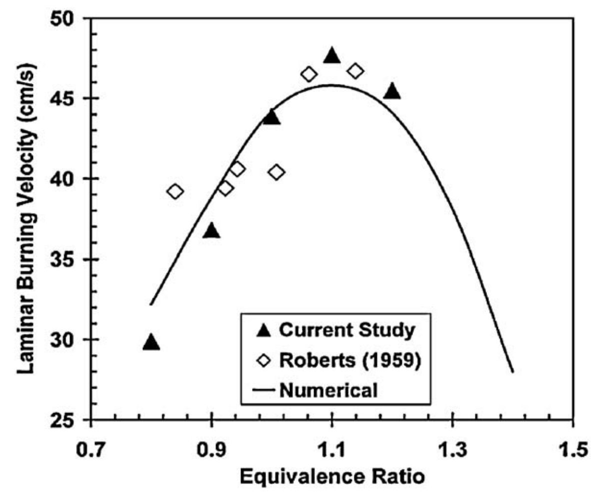


Fig. 6. Laminar burning velocities of butanol/air mixtures as the temperature is 350 K and the pressure is 0.89 atm [114].

Recently, McEnally and Pfefferle measured temperature and species in atmospheric pressure coflowing laminar non-premixed methane flames doped with one of four isomers of butanol (including n-butanol) [110]. They concluded that unimolecular dissociation dominates over H-atom abstraction. This consisted of C–C fission followed by β-scission of the resulting radicals. Yang et al. [111] investigated low-pressure laminar premixed flames fueled by one of four isomers of butanol (including n-butanol) using synchrotron radiation photoionisation and molecular beam mass spectrometry to identify transient and stable species. Their results, although not quantitative, are extremely valuable from the viewpoint of mechanism construction. Dagaut et al. [112] reported new experimental data obtained in a jet stirred reactor (JSR) for the oxidation of n-butanol at 1013.25 kPa, equivalence ratios spanning 0.5–2.0, and the temperature ranging 800–1150 K. The authors also presented a novel chemical kinetic mechanism that provided good overall agreement with the experimental data. The validated mechanism indicates that H-abstraction reactions are the main consumption pathway for n-butanol in the JSR at 10 atm. Dagaut and Togbé [113] studied the oxidation of butanol–gasoline surrogate mixtures (85–15 vol.%) in a JSR at 10 atm, equivalence ratios spanning 0.3–2.0, and temperatures ranging from 770 to 1270 K. The surrogate mixture comprised of iso-octane, toluene, 1-hexene and n-butanol. A novel chemical kinetic mechanism was derived using mechanisms for each pure component in the butanol–gasoline surrogate mixture, and it was shown to provide good agreement with the experimental data. Similar to the aforementioned JSR study of pure n-butanol, the mechanism indicates that H-abstraction reactions are the main consumption pathways for the butanol–gasoline surrogate fuel. Sarathy et al. [114] presented new experimental data for n-butanol in three experimental configurations. Their experimental results indicated laminar burning velocity increases between ϕ = 0.8 and 1.1, which corresponds to a maximum burning velocity of 47.7 cm/s, and then decreases at higher equivalence ratios, as shown in Fig. 6. The proposed model verified by the JSR and opposed-flow diffusion flame indicated that H-abstraction is the major pathway for n-butanol consumption, followed by β-scission of the resulting fuel radicals. Moss et al. [115] studied the autoignition of the four isomers of butanol in a shock tube at temperatures and pressures ranging from 1200 to 1800 K and 1–4 bar. A detailed kinetic mechanism was developed to describe the oxidation of the butanol isomers and was validated

by comparison to the shock tube measurements. Results indicated that the consumption of n-butanol takes place primarily by H-atom abstraction resulting in the formation of radicals. Black et al. [116] measured the autoignition delay time at equivalence ratios of 0.5, 1 and 2 for n-buatnol at reflected shock pressures of 1, 2.6 and 8 atm at temperatures from 1100 to 1800 K. Results showed H-abstraction to be the principal route of consumption of butanol, and abstraction from the α position dominates, followed by the γ, β and δ positions, while abstraction from the hydroxyl group is of much lesser importance. By comparison, unimolecular decomposition accounts for only 25% of butanol consumption. Gu et al. [117] investigated the laminar burning velocities of n-butanol–air premixed mixtures by using the spherically expanding flame at different initial pressures (1–2.5 bar) and temperatures (413–473 K) over a wide range of equivalence ratios (0.8–1.6). They found that the laminar burning velocities decrease with the increase of initial pressure, while the laminar burning velocities increase as initial temperature increases. The flame thickness is decreased remarkably with the increase of initial pressure.

4.1.2. Summary and future directions in fundamental combustion

It can be concluded from aforementioned review that the consumption of n-butanol includes two pathways. One is that unimolecular dissociation dominates over H-atom abstraction. The other is that H-abstraction is the major pathway for n-butanol consumption. These findings are different because of differences in experimental setups. Barnard suggested that fission at the C_3H_7–CH_2OH bond to produce the n-propyl radical and hydroxymethyl radical was dominant [108]. Barnard's findings are consistent with what one would expect from a pyrolysis study where oxygen is absent, hence unimolecular dissociation dominates. McEnally and Pfefferle studied coflowing laminar non-premixed methane flames doped with n-butanol, and concluded that unimolecular dissociation dominated over H-atom abstraction [110]. Their findings are logical since it applies to a doped coflow flame in the centerline region, where the temperatures are very high (>1300 K), the fuel concentration is at a maximum, and the concentrations of radical species is at a minimum. It is not clear if the proposed unimolecular decomposition mechanism would dominate in coflow flames of pure n-butanol (i.e. undoped flames). H-abstraction dominates in the experimental configurations presented herein due to the autoignition processes [111–116]. The JSR and shock tube are premixed apparatus so there is a rapid formation of radical species, which accounts for H-atom abstraction reactions being dominant. H-atom abstraction dominates in the opposed-flow diffusion flame because radical species generated near the flame front are able to diffuse into the fuel stream to consume the fuel. Unimolecular decomposition is not significant even at regions where radical species concentrations are small because the temperature of the fuel stream is low at these points.

Studies on the fundamental combustion of the n-butanol are summarized in Table 8. The burning velocity of the n-butanol has been reported by several groups because it is one of the key parameters that determine the propagation and stabilization of premixed flames. Meantime, the premixed and non-premixed flames have been studied in a wide temperature range, from 530 to 1800 K, covering the low temperature oxidation and high temperature oxidation, the ambient pressures spanning 1–10 atm, and the fuel–air equivalence ratios ranging from 0.25 to 2.0. In addition, the detailed chemical kinetics mechanism of n-butanol has been proposed and validated by some research groups. However, some studies on the fundamental combustion of the n-butanol are still needed in several aspects as suggested by the following:

For a diesel engine operating condition, the ambient pressure can reach 40 atm as the autoignition occurs and the equivalence ratio in some rich zones can exceed 3. Therefore, there is a need for validating models in a higher equivalence ratio, i.e. above 3 and in a higher ambient pressure to mimic a real diesel engine condition.

As summarized in Table 8, only a simple diesel fuel surrogate (n-heptane) and a gasoline surrogate (iso-octane, toluene, 1-hexene) were studied by Dagaut and Togbé [113,118] to develop the chemical kinetics mechanism of the butanol–gasoline or butanol–diesel blends. Accordingly, there is a need for more experimental studies and chemical kinetic models of the butanol blends. Furthermore, the surrogate of gasoline or diesel fuel should be more complicated and more similar to the real fuel, i.e. adding cycloalkanes and aromatics for a diesel surrogate.

Some researches on spray, combustion, and emissions of n-butanol are needed in some optical engines, rapid compression machine (RCM), constant volume chamber (CVC), etc., to mimic a more real spray and combustion processes in gasoline or diesel engines. And these studies are also very important to the computational fluid dynamics (CFD) development in spray and combustion process. Only a few studies on the n-butanol are carried out in these conditions, which will be reviewed in Sections 4.2.2 and 4.3.3.

4.2. A substitute for gasoline in SI engines

As aforementioned, butanol is less likely to separate from the blend fuel than ethanol if the fuel is contaminated with water. This facilitates storage and distribution of blended fuels. Butanol doubles the carbon of ethanol and contains 25% more energy, therefore the fuel consumption rate will be lower for butanol than for ethanol. It can be used alone in an internal combustion engine, or it can be mixed with gasoline without modification. Because of these advantages, there has been increasing number of studies on butanol in SI engines or in cooperative fuel research (CFR) engines.

4.2.1. Studies of n-butanol in the cooperative fuel research (CFR) engines

Investigations of n-butanol in CFR engines have been conducted by several research groups. Yacoub et al. [121] performed several studies on application of straight-chain alcohols C_1–C_5 (methanol to pentanol) as fuels blended with gasoline in the CFR engine at 1000 rpm, stoichiometric ratio. The engine operating conditions were optimized for each blend with two different values of matched oxygen mass content (2.5% and 5.0%), corresponding to 11% and 22% volume content for the n-butanol. Their studies showed that n-butanol was more prone to generate combustion knock than unleaded test gasoline (UTG) with RON = 96. All alcohol–gasoline blends showed reduction in CO emissions, and THC emissions were also reduced at optimized operating conditions. However, all blends had a higher unburned alcohol emission than gasoline, and the unburned alcohol emission was higher for blends with higher content of alcohol. Aldehyde emissions were higher for all blends with formaldehyde as the main constituent, and the NO_x emissions may increase or decrease depending on different operating conditions.

Another research in the CFR engine was conducted by Gautam and Martin [122,123] using six alcohol–gasoline blends at 900 rpm and stoichiometric ratio. Each blend contained 90 vol.% gasoline (UTG-96) and 10 vol.% alcohol. The alcohol component used in the blend included methanol, ethanol, propanol, butanol and pentanol, and the concentrations of individual alcohols in the mixture were varied. Results showed the higher the oxygen content in the blend, the higher the knock resistance and the faster the flame speed. Emissions tests at maximum power operating condition showed that the brake specific emissions were significantly lower for the higher alcohol content than for neat gasoline (16–20% lower CO, 18–23% lower CO_2, 5–11% lower NO_x, and 17–23% lower organic material hydrocarbon equivalent). This is because the blends have greater resistance to knock and allowed higher compression ratios,

Table 8
Studies on the fundamental combustion of the n-butanol.

Fuel	Experimental apparatus	Ambient temperature	Ambient pressure	Fuel–air equivalence ratio	Chemical kinetic model
n-Butanol 100% [110]	A burner generated coflowing laminar non-premixed flames	–	1 bar	–	No chemical kinetic model was proposed
n-Butanol 100% [111]	A flat-flame burner generated a low-pressure laminar premixed flame	2200 K (flame temperature)	4 kPa (gas pressure)	1.71 (fuel–oxygen equivalence ratio)	No chemical kinetic model was proposed, but species in n-butanol flame were identified by using synchrotron radiation photoionisation and molecular beam mass spectrometry
n-Butanol 100% [112]	JSR generated a full premixed flame	800–1150 K	10 atm	0.5–2.0	A novel detailed chemical kinetic mechanism was proposed, and it can predict the combustion species getting from gas chromatographs very well
Mixtures of n-butanol and gasoline surrogate (85–15 vol.%) [113]	JSR generated a full premixed flame	770–1270 K	10 atm	0.3–2.0	A novel chemical kinetic mechanism (251 species, 1990 reversible reactions) was derived using mechanisms for each pure component in the butanol–gasoline surrogate mixture, and it provides good agreement with the experimental data from gas chromatographs and on-line fourier transform infrared (FTIR) analysis
n-Butanol 100% [114]	1. JSR generated a full premixed flame 2. Two identical flat-flame burners facing each other generated opposed-flow diffusion flame 3. A cylindrical combustion chamber was used to measure the laminar flame speed	800–1250 K for the JSR; 350 K for the laminar flame speed measurement	1 atm for the JSR; 0.89 atm for the laminar flame speed measurement	0.25–2.0 for the JSR measurement 0.8–1.2 for the laminar flame speed measurement	An improved detailed chemical kinetic mechanism (118 species, 878 reactions) based on Dagaut et al. [112] was proposed, and it provides good agreement with the experimental data from premixed and diffusion flame
n-Butanol 100% [115]	A shock tube generated a full premixed flame	1200–1800 K	1–4 bar	0.25–1	A detailed kinetic mechanism has been developed to describe the oxidation of the n-butanol and validated by comparison to the shock tube measurements
n-Butanol 100% [116]	A shock tube generated a full premixed flame	1100–1800 K	1–10 atm	0.5–2	A detailed chemical kinetic model was constructed and used to simulate the ignition delay data, with good agreement. And it provide good agreement with the experimental data from Dagaut et al. [112]
n-Butanol 100% [117]	A constant volume combustion chamber generated a premixed laminar flame	413–473 K	1–2.5 bar	0.8–1.6	No chemical kinetic model was proposed
n-Butanol and n-heptane (20/80 and 50/50 in mol) [118]	JSR generated a full premixed flame	530–1070 K	10 atm	0.5–1	A kinetic modeling was performed using reaction mechanisms resulting from the merging of validated kinetic schemes for the oxidation of the components of the n-heptane and n-butanol
n-Butanol 100% [119]	A spherical closed pressurized combustion vessel generated a premixed laminar flame	373 K	10 bar	0.8–1.3	A chemical kinetic mechanism of n-butanol from Dagaut et al. [112] was used to calculate laminar burning velocity
n-Butanol 100% [120]	A counterflow burner generated a premixed laminar flame	343 K for the unburned mixture temperature	1 atm	0.7–1.5	Two recently developed detailed kinetics models for the oxidation of n-butanol were used for numerical simulations of laminar flame speeds

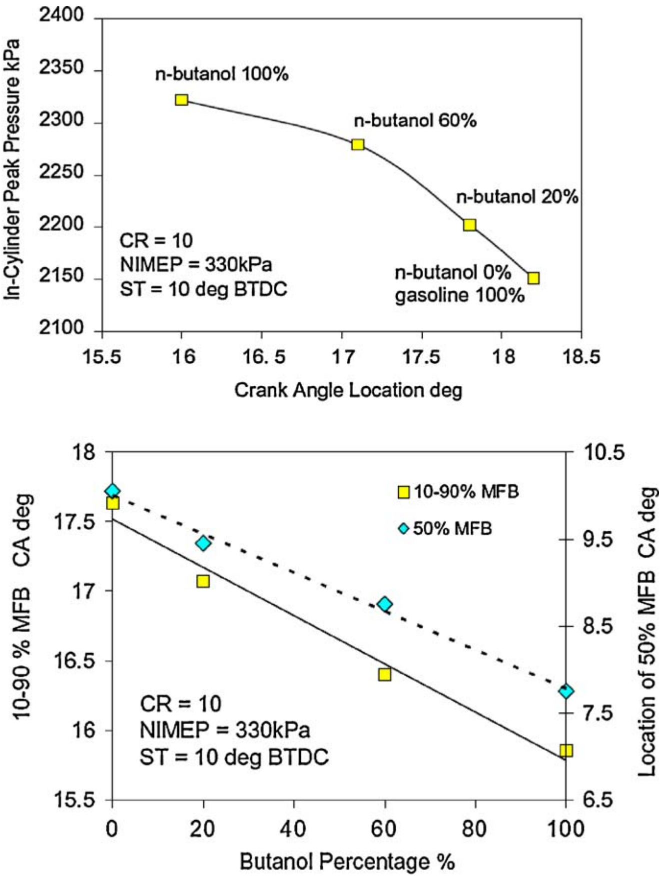


Fig. 7. Location of in-cylinder peak pressure, 10–90% MFB, and 50% MFB for n-butanol–gasoline blends [124].

which increase engine power output. Thus, the brake specific emissions were lower. Cycle fuel consumption (mass per unit time) of blends with higher alcohol content was 3–5% higher than neat gasoline, however, the BSFC for the blends was 15–19% lower than the BSFC of neat gasoline.

Szwaja and Naber [124] investigated the combustion characteristics of the n-butanol in a single cylinder CFR engine by changing the spark timing (from 18° to 4° BTDC), n-butanol volumes (from 0% pure gasoline to 100% pure n-butanol, the pump octane number (PON) of the gasoline is 87), compression ratios (from 8 to 10), and loads (3.3 and 6.5 bar indicated mean effective pressure (IMEP)) at 900 rpm, using stoichiometric ratio. Results indicated that the highest peak pressure advanced with the increase of n-butanol ratio due to a faster combustion and the crank angle degree (CAD) of 50% mass fraction burn (MFB) for n-butanol was approximately 2° earlier when compared to gasoline, as illustrated in Fig. 7. Therefore, the optimal spark timing (to obtain maximum brake torque (MBT)) for pure n-butanol–air stoichiometric mixture should be retarded. The behavior of neat n-butanol with respect to combustion knock is similar to that of PON 87 gasoline, which includes the sensitivity to spark timing and compression ratio. Through the experimental data, the authors demonstrated that n-butanol can directly substitute gasoline either as a neat fuel or as fuel blends from the perspectives of combustion, energy density, and similar thermo-physical properties.

4.2.2. n-Butanol studies in some SI engines

There have also been studies on applications of butanol in SI engines. Alasfour [125] investigated the effect of using 30% volume butanol–gasoline blend on the first- and second-law efficiency in a single-cylinder SI engine at ϕ ranging from 0.8 to 1.2. The engine second-law efficiency shows a reduction of 7% when using gasoline–butanol blend compared to pure gasoline fuel at $\phi = 0.9$. Dernotte et al. [126] evaluated the combustion and emissions characteristics of butanol gasoline blends in a port fuel injection, SI engine. The main results demonstrated that a 40% butanol/60% gasoline blend by volume minimized HC emissions and no significant change in NO_x emissions were observed with the exception of the 80% butanol/20% gasoline blend. The addition of butanol improved combustion stability, as measured by the coefficient of variation (COV) of IMEP, and reduced ignition delay (0–10% MFB). The change of specific fuel consumption of B40 blend was within 10% of that of pure gasoline for stoichiometric mixture.

Wallner et al. [33] investigated the combustion, performance, and emissions of pure gasoline, 10% ethanol (E10) and 10% butanol (Bu10) blends in a direct-injection (DI) four-cylinder SI engine with 220 Nm of maximum torque. The engine was operated at speeds from 1000 to 4000 rpm with loads starting from 0 Nm (idle) up to 150 Nm. Results showed that the burning velocity of the Bu10 was higher than those of both the E10 and gasoline. Combustion stability did not vary significantly between the three fuels, and the COV of IMEP was less than 3% for the entire operating range. The Bu10 and neat gasoline are more prone to knock at high load compared with the E10. BSFC showed an increase of approximately 3.4% for Bu10 and 4.2% for E10 compared with gasoline, while the brake thermal efficiency was very similar between the three fuels. Specific CO and HC emissions did not show a significant difference between gasoline and the two fuel blends. The specific NO_x emissions were the lowest for Bu10, which retarded spark timing at the high load conditions due to the low octane rating of pure butanol. Based on experimental results, they found that 10 vol.% butanol can be substituted for ethanol with an improvement in fuel economy and no degradation in emission or combustion stability. Their further study [127] reported the regulated and non-regulated emissions from the combustion of gasoline, alcohol fuels and their blends in the same engine. Gasoline was used as a baseline fuel and compared to gasoline–ethanol blends at volumetric blending ratios of 10% and 50%. n-Butanol and iso-butanol were tested at volumetric blend ratios of 16% and 83% which correspond to E10 and E50 in terms of oxygen content. Results demonstrated that addition of alcohol to the fuel blend results in a consistent reduction in NO_x emissions regardless of operating point. Both formaldehyde and acetaldehyde emissions increase with addition of butanol to the blend, whereas formaldehyde does not increase significantly with addition of ethanol, as illustrated in Fig. 8. Propene, 1,3-butadiene, and acetylene emissions, which are required for carbon growth processes leading to benzene, also increase only with addition of butanol, as illustrated in Fig. 8. Because benzene formation appears to be rate-limiting in soot formation, PM is expected to be significantly higher in emissions from engines burning butanol blends as compared to blends of ethanol. Therefore, although butanol has many clear advantages over ethanol as a fuel to meet current and future emission regulations, burning this fuel at high blend ratios may result in an increased level of non-regulated emissions.

Yang et al. [128] discussed the feasibility of fueling gasoline engines with butanol–gasoline blends, ranging from 10% to 35% butanol by volume. Test results showed the engine power can be maintained when the butanol concentration is below 20%. The maximum engine power drops and BSFC goes up slightly when the concentration of butanol approaches 30%, but those could be recovered by optimizing engine operational parameters. In all the cases investigated engine raw HC and CO emissions are significantly reduced but NO_x emissions go up.

Cairns et al. [129] evaluated the combustion and emissions of the gasoline, gasoline–ethanol, and gasoline–butanol blends in a turbocharged multi-cylinder DI–SI engine equipped with an external exhaust gas recirculation (EGR) circuit. Results indicated that

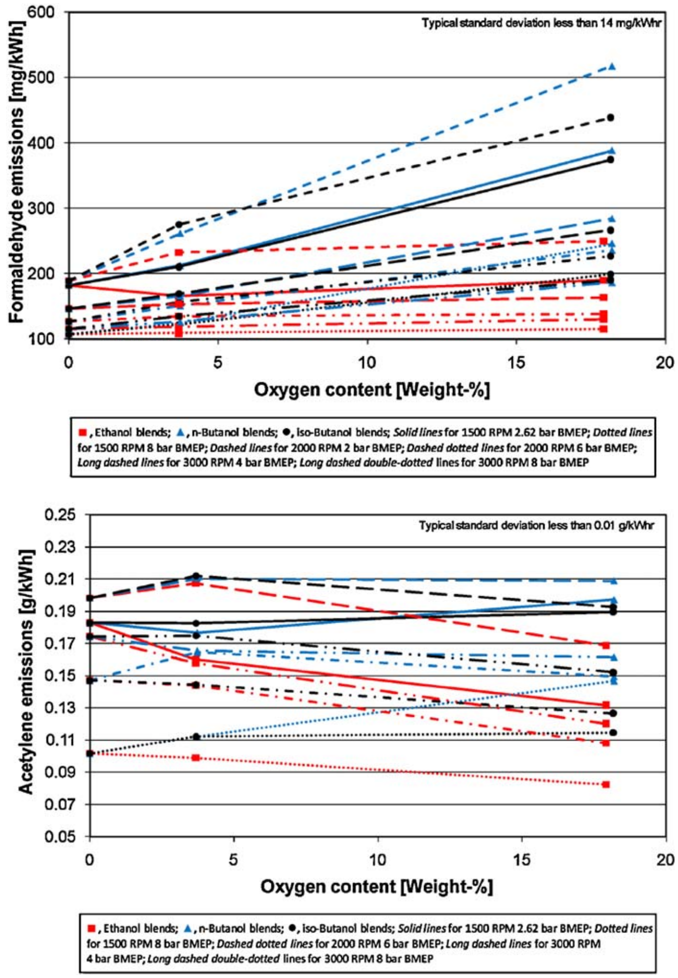


Fig. 8. Formaldehyde and acetylene emissions as a function of operating point and oxygen content for ethanol, n-butanol and iso-butanol blends [127].

the 10% ethanol and 16% butanol fuels with the similar oxygen content produced similar values of BSFC and similar MBT spark timings at the same conditions. In their further study [130], the effects of alcohol blends on combustion, fuel economy and emissions were conducted in a single cylinder engine equipped with a mechanical fully variable valvetrain on the inlet and variable valve timing on the exhaust. A number of blends of gasoline, iso-octane, ethanol and butanol were examined during port fuel injected early inlet valve closing operation, both with and without variable valve timing. Under low valve overlap conditions, it was apparent that the inlet valve durations/lifts required for full unthrottled operation were remarkably similar for the wide range of blends studied.

Williams et al. [131] evaluated the maximum achievable thermal efficiency of a SI engine using gasoline and a range of and advanced biofuels, including isomers of butanol. Engines used in this study included an Atkinson cycle 1.5 L and prototype 1.8 L lean boosted engine. This study was aim to establish practical and implementable routes to minimize well-to-wheels CO_2 emissions. Results indicated that thermal efficiency, combustion and emissions were not adversely affected as a result of adding butanol to gasoline. The use of fuels containing high octane bio-components coupled with future engine technologies, such as downsized lean-boosting, will lead to a reduction in engine CO_2 emissions. Further CO_2 savings can be achieved by increasing the H/C ratio of the fuel.

Aleiferis et al. [132] reported the spray development and combustion of ethanol, butanol, iso-octane, and gasoline in a DI–SI optical engine at 1500 rpm, stoichiometric ratio, part load (0.5 bar intake pressure), with injection timing set early in the intake stroke to promote homogeneous mixture formation. The effects were investigated at engine coolant temperatures of 20 and 90 °C. They found the spray developments of butanol were similar at both 20 and 90 °C but with small changes in spray plume geometries apart from wider plumes and increased effect of air entrainment into the spray plumes at 90 °C, while the ethanol and gasoline sprays were seen to partially collapse and produce a spray 'cloud' with a footprint directly below the injector nozzle at 90 °C, as illustrated in Fig. 9. The growth rates of butanol spray areas were the lowest among these four fuels. Butanol flame development was very similar to that of gasoline at both 20 and 90 °C engine coolant temperatures, with a marginally faster main period of flame development relative to gasoline.

4.2.3. Summary and future directions in SI engines

Based on aforementioned reviews, it can be found that these researches have covered some different types of SI engines, such as CFR engine, optical engine, and some single- or multi-cylinder engines. Some of these engines are equipped with advanced technologies, i.e. turbocharger, fully variable valvetrain, direct-injection. All these studies have shown some similar results as follows:

4.2.3.1. Combustion. The knocking tendency of n-butanol is similar to that of PON 87 gasoline, which includes the sensitivity to spark timing and compression ratio. Combustion durations for pure n-butanol and n-butanol blends are comparable to those for gasoline. n-Butanol blends reduce the ignition delay (0–10% MFB) and advance the CAD of 50% MFB compared with the gasoline. To obtain the maximum break torque, the spark ignition timing should be retarded when using the butanol blends due to their faster combustion. Combustion stability does not vary significantly for the pure n-butanol or butanol blends as measured by the COV of IMEP.

4.2.3.2. Performance. The engine power can be maintained without any necessary modifications to the engine when butanol content is below 20% by volume, while the maximum engine power drops as the blending ratio of butanol approaches 30% by volume. With the increase of n-butanol content in the blends, the specific fuel consumption will increase due to its lower energy density compared with the neat gasoline. However, there is only a little penalty for the BSFC when n-butanol content is below 20%, while the change in specific fuel consumption is within 10% of that of pure gasoline for stoichiometric mixture when n-butanol content goes up to 40% in the blend. n-Butanol blend has lower specific fuel consumption than the ethanol blend due to its higher heating value.

4.2.3.3. Emissions. The CO, THC, and NO_x emissions may be reduced or increased depending on the specific engine (i.e. port-injection or direct-injection), operating conditions (i.e. with or without closed-loop air–fuel ratio control, spark timing), and blending ratios. Butanol blends have a higher unburned alcohol emission rates than gasoline, and the unburned alcohol emission rates are higher for blends with higher content of n-butanol. Aldehyde emissions are higher for the n-butanol blends with formaldehyde as the main constituent, whereas formaldehyde does not increase significantly with addition of ethanol. Some compounds which are required for carbon growth processes leading to benzene, increase with the addition of butanol compared with the neat gasoline or ethanol blend. Because benzene is a precursor of soot, a higher soot emission from the DI–SI engine burning butanol blends is expected.

Based on aforementioned reviews, it can be found that the reports of the n-butanol are not as extensive as researches on

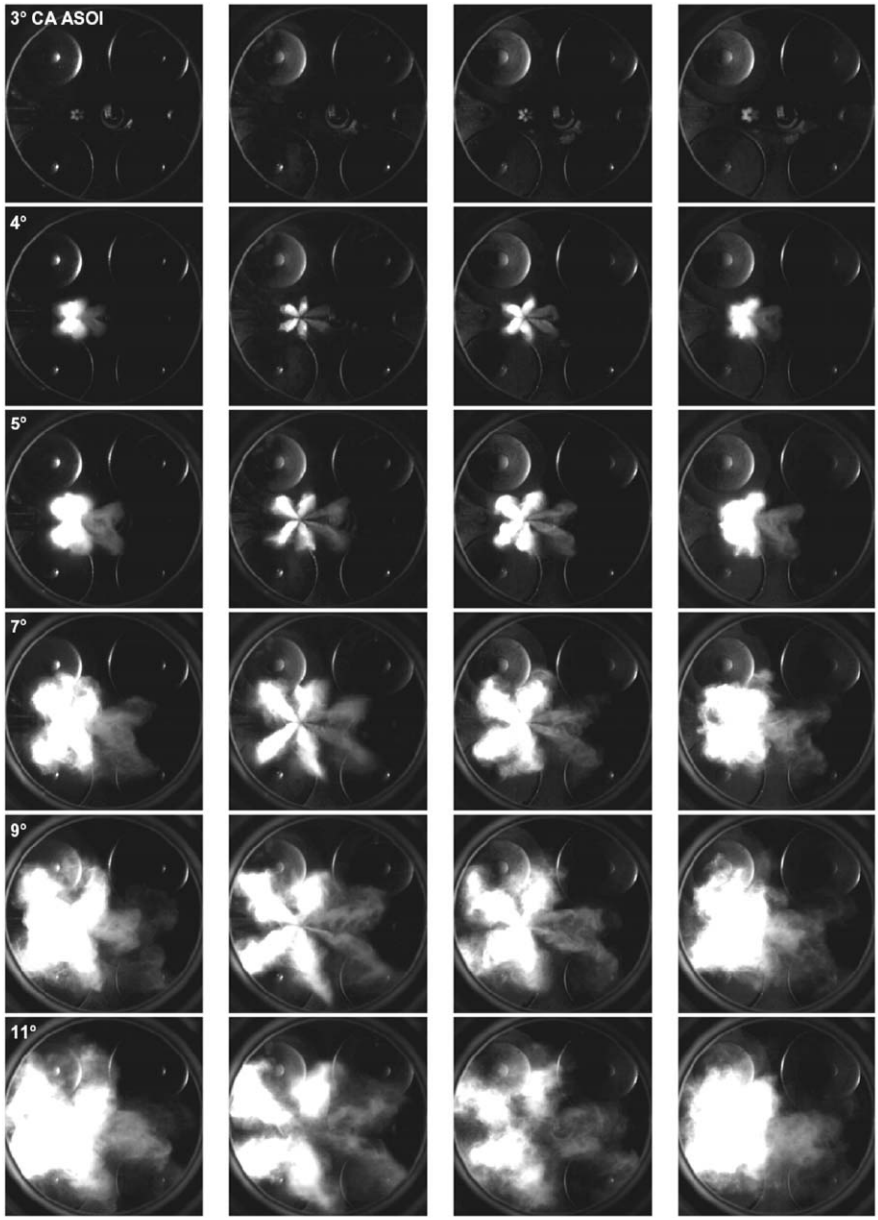


Fig. 9. Spray development: column 1 ethanol, column 2 butanol, column 3 iso-octane, column 4 gasoline, using SOI 80 °CA ATDC under motoring conditions at 1500 rpm, 90 °C engine coolant temperature; each image is labeled by its crank angle timing following the start of injection, i.e. °CA after start of injection (ASOI) [132].

ethanol blends. Therefore, more studies of n-butanol are needed in several aspects:

1. More extensive studies should be conducted on the different engine types, i.e. engines with low or high mileage usage, because the characteristics of combustion, performance, and emissions in these used engines might be different from new or experimental engines.
2. More research on emissions, including steady and transient measures, CO_2 emissions with well-to-wheel, and some regulated and non-regulated emissions should be carried out. This will provide a deeper insight on the emission mechanisms and will lead to a more comprehensive understanding of the advantages and disadvantages of butanol as an alternative fuel.
3. Few reports can be found regarding the combustion and emissions of n-butanol blends tested with new combustion technologies, such as the controlled autoignition (CAI). Since the CAI combustion is one of the potential technologies capable of helping meet more stringent emission regulations in the future, more researches should be conducted with CAI combustion fueling pure butanol or butanol blends.
4. The studies on the spray and combustion of the butanol using optical diagnostics are not extensive in optical engines, rapid compression machines, or some constant volume combustion chambers, while these optical diagnostics are very important to revealing the spray and combustion characteristics in more real gasoline engine conditions, and they are also important to the development of CFD models.

4.3. A substitute for diesel fuel in compression ignition (CI) engine

Biodiesel, which has many similar properties like the diesel fuel and can be blended with diesel in any proportion, has been intensively studied and widely used in diesel engines. Meanwhile,

Table 9
Properties of diesel, butanol, and biodiesel [16,134–137].

	Diesel	Biodiesel	n-Butanol
Molecular formula*	C_{12}–C_{25}	C_{12}–C_{24}	C_4H_9OH
Cetane number	40–55	47–52	25
Oxygen content (% weight)	–	10	21.6
Density (g/mL) at 20 °C	0.82–0.86	0.87	0.808
Autoignition temperature (°C)	246	363	385
Flash point (°C) at closed cup	65–88	166	35–37
Low heating value (MJ/kg)	42.5	38.81	33.1
Boiling point (°C)	180–370	262–359	117.7
Stoichiometric ratio	14.3	12.5	11.21
Latent heating (kJ/kg) at 25 °C	270	200	582
Viscosity (mm^2/s) at 40 °C	1.9–4.1	4.0	2.63

* For the diesel and biodiesel, only gives the carbon numbers.

alcohols, mainly ethanol and to a much less extent methanol, have also been considered as alternative fuels for diesel engines [3,11,19,20,26]. However, there are some drawbacks when using ethanol–diesel or methanol–diesel blend in the diesel engines [133], i.e. increased risks of fire and explosion compared to diesel fuel or even to gasoline due to the vapor pressure and flammability limits of ethanol, decreased maximum power, increased incidence of fuel pump vapor lock, and reduced fuel pump and fuel injector life due to decrease lubricity of ethanol. In addition, because phase separation between ethanol or methanol and diesel remains a problem, cosolvents are needed when using these alcohol–diesel blends.

Butanol can be a future option for blending with diesel and can overcome some drawbacks of ethanol as reviewed in Section 2.2. Therefore, we only compare the properties of biodiesel and butanol in this section, which are listed in Table 9 [16,134–137]. Compared with biodiesel, butanol contains more oxygen content, which can reduce the soot emission further. The NO_x emissions can also be reduced because its higher heat of evaporation results in a lower combustion temperature [32]. In addition, the viscosity of butanol is also more suitable for diesel engine than that of biodiesel. The drawback of n-butanol should be a lower cetane number compared with diesel or biodiesel fuels. However, a lower cetane number may be a need in future diesel combustion to increase autoignition delay and form more premixed combustion, thereby reducing the soot emissions.

4.3.1. n-Butanol–diesel blend properties

Chotwichien et al. [138] investigated the stabilization of ethanol–diesel and butanol–diesel blends using the biodiesel as a cosolvent. At temperatures of 10–30 °C, the miscibility of diesel–biodiesel–butanol was higher than the miscibility of diesel–biodiesel–ethanol, and the use of butanol in diesohol could solve the problem with phase separation because of more solubility in the diesel fuel. Further, the blends of diesohol containing butanol have closer properties to diesel than the blends of diesohol containing ethanol. The blend of 85% diesel, 10% palm oil ethyl ester, and 5% butanol was the most suitable, as it provided a stable mixture and acceptable fuel properties for use as an alternative fuel in diesel engines.

Another study on the butanol–diesel blends properties was carried out by Zoldy et al. [139]. To check the application properties of butanol–diesel mixtures, more than 20 chemical and physical parameters of blends were tested including flash point, cetane number, viscosity and cold filter plugging point. The effects of butanol blending were also tested on power, fuel consumption and injector cleanness. Results demonstrated that low butanol blends (up to 5 vol.% content) appear to be a good alternative for compression ignition engine utilization: they hold the cetane number limits without extra cetane booster additive; they allow nearly the same fuel consumption level along with a considerable increase in injector cleanness. 10 vol.% butanol has the app. 2% higher fuel consumption as measured in engine dynamometer and calculated from theoretical heating value. This 2% is in the same range as the approximately 1.8% increase in fuel consumption increase observed in 10 vol.% biodiesel blend.

4.3.2. n-Butanol studies in some CI engines

Investigation of n-butanol usage as the CI engine fuel has been conducted by several research groups. Yao et al. [140] investigated the influence of n-butanol–diesel blend on the performance and emissions of a heavy-duty direct-injection diesel engine with multi-injection and various EGR ratios. At specific engine speed (1849 rpm) and load (11.6 bar break mean effective pressure), EGR rates were adjusted through a variable nozzle turbocharger to keep NO_x emission at 2.0 g/kW h. Diesel fuels with different contents (0%, 5%, 10% and 15% by volume) of n-butanol were used. Results demonstrated that the in-cylinder pressures do not show much difference for various blends, whereas higher n-butanol fraction in the blend results in higher premixed combustion heat release. The soot and CO emissions can be improved by the n-butanol addition without a serious impact on the break specific fuel consumption. The impacts of pilot and post injection on engine characteristics by using blended fuels are similar to that found by using pure diesel. As shown in Fig. 10, early pilot injection reduces soot emission, but results in a dramatic increase of CO, while post injection reduces soot and CO emissions effectively. Under each injection strategy, the increase of fuel n-butanol content leads to further reduction of soot. A triple-injection strategy with the highest n-butanol fraction (15% vol.) offers the lowest soot emission. In addition, Yao et al. [141] also studied the effect of EGR ratio on the performance

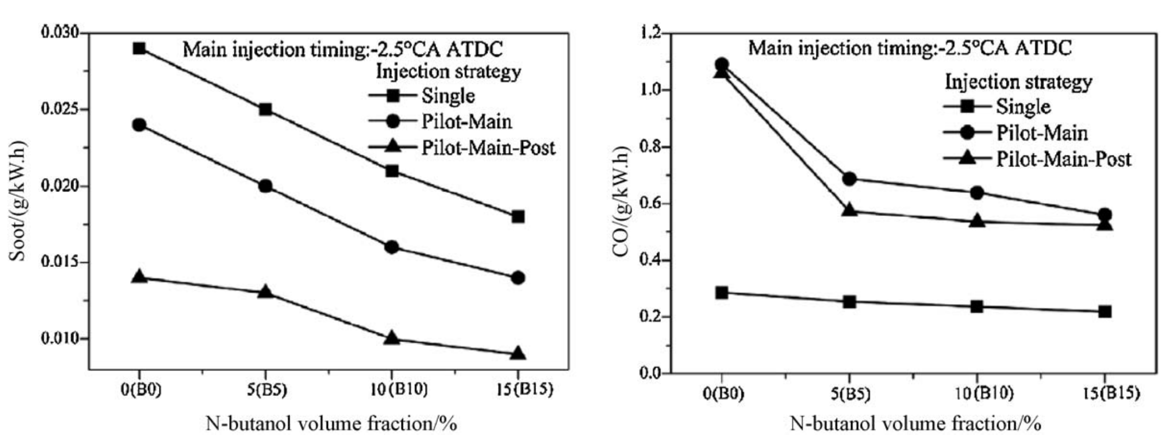


Fig. 10. Comparison of soot and CO emissions between various fuels and injection strategies [140].

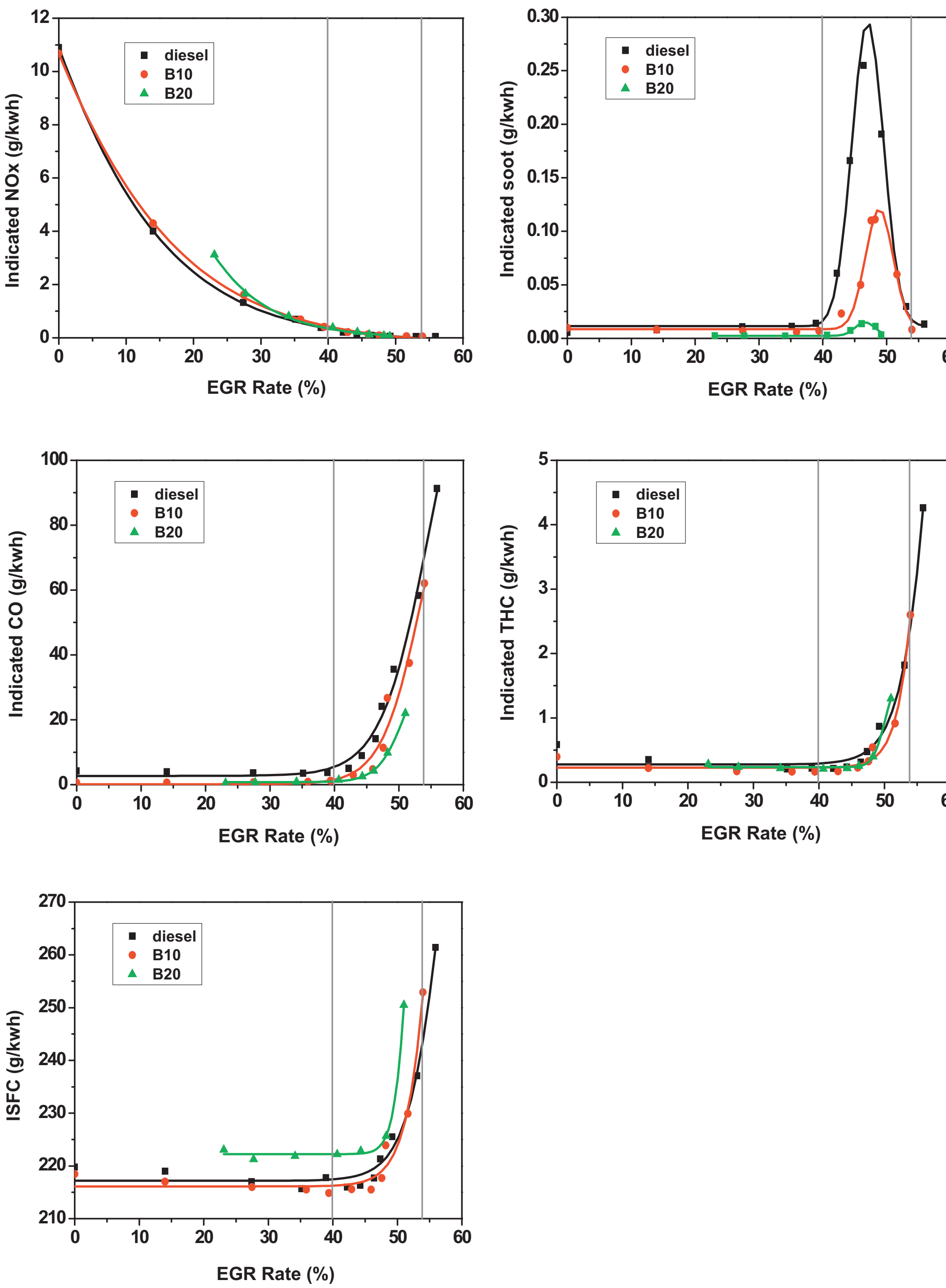


Fig. 11. Effects of butanol blend ratios on NO_x, Soot, CO, and THC emissions and indicated specific fuel consumption (Engine operating conditions: Speed: 1400 rpm, Compression ratio: 16, Boost: 1.5 bar, Start of injection: −10° ATDC, Injection mass: 50 mg/cyc, Injection pressure: 1600 bar) [141].

and emissions in a heavy-duty common-rail diesel engine using n-butanol and diesel blends at volumetric blending ratios of 10% (B10) and 20% (B20). It was found that butanol–diesel blend can reduce the soot emission dramatically during the medium EGR range (∼40–54% EGR ratio) and have the potential to decrease NO_x emissions due to allowing higher EGR rates without increasing soot emissions. In addition, n-butanol addition reduced the CO emission, and had little effect on THC emissions. The 10% butanol blend had little effect on the indicated specific fuel consumption (ISFC), while the 20% butanol blend increased the fuel consumption about 2% at the EGR ratio below 50%. Therefore, n-butanol additive can endure a higher EGR ratio without the penalty of HC, CO, soot emissions, and ISFC, as illustrated in Fig. 11.

Rakopoulos et al. [32] investigated the effects of using blends of n-butanol and diesel fuel with 8%, 16% and 24% (by volume) n-butanol, on the performance and exhaust emissions of a high speed diesel engine. The tests are conducted at a speed of 2000 rpm and three different loads of 1.4, 2.57, 5.37 bar BEMP (break effective mean pressure). Experimental data demonstrated the soot was significantly reduced with the use of the butanol–diesel blends, and the blends had a larger reduction for soot under high load, as shown in Fig. 12. Similar trends were also illustrated in their further research [142] in a heavy-duty diesel engine and the report of Liu et al. [143] in a constant volume chamber. These studies have indicated the fuel-bound oxygen has a dominant influence in locally rich zones. Compared with the neat diesel fuel, the NO_x and CO

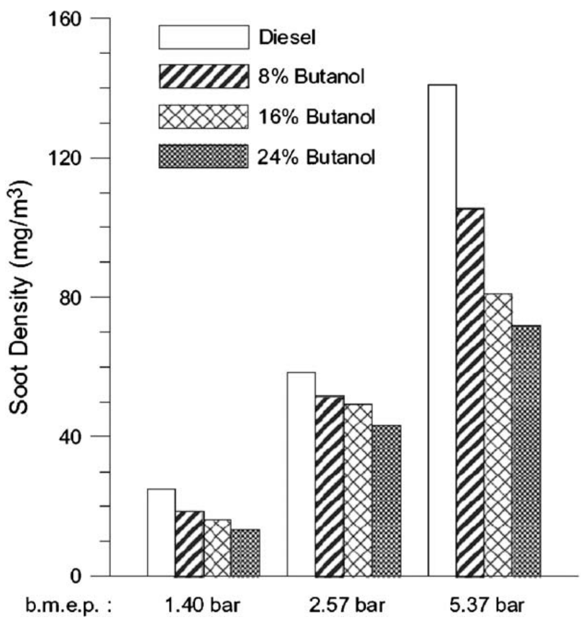


Fig. 12. Emitted soot density at the three loads using the neat diesel fuel and the 8%, 16% and 24% butanol blends [29].

emissions were reduced, while the HC emissions were increased with the use of the butanol–diesel blends. Increasing the butanol blending ratio leads to a little higher specific fuel consumption, while the thermal efficiency increases slightly and exhaust gas temperatures reduce a little compared with the neat diesel fuel. The higher thermal efficiency can be attributed to the larger portion of premixed combustion and to the lower heat losses. The ignition delay of the butanol blend is higher than that of the neat diesel fuel, therefore its premixed combustion peak is much higher and 'sharper' as illustrated in Fig. 13, leading to a higher percentage of 'constant volume' combustion. The lower heat losses are attributed to the lower in-cylinder temperature because of a higher latent heating value for butanol blends. Based on the experimental results, the Rakopoulos et al. [29,142] research on the light- and heavy-duty diesel engine showed that n-butanol can be used safely and advantageously up to high blending ratios (24% vol.) with the diesel fuel in CI engine.

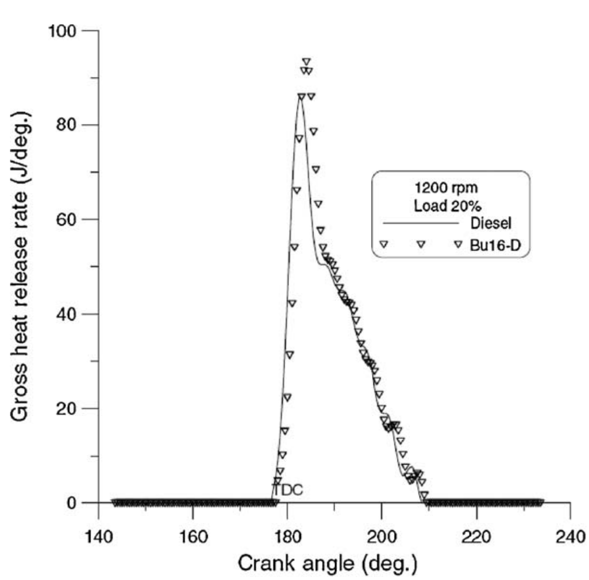


Fig. 13. Gross heat release rate for the neat diesel fuel and the 16% n-butanol blend at 1200 rpm [142].

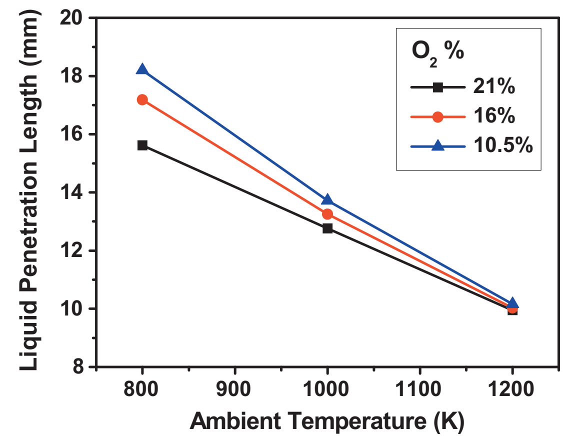


Fig. 14. The liquid penetration length at various ambient temperatures and oxygen concentrations [146].

Miers et al. [144] investigated the engine performance and emissions in a 1999 Mercedes Benz C220 turbo diesel vehicle (Euro III compliant) fuelling 20% and 40% by volume blends of butanol with diesel fuel. The results showed that for the urban drive cycle, both THC and CO emissions increased as larger quantities of butanol were added to the diesel fuel. NO_x were not significantly affected by the 20% butanol blend and decreased with the 40% butanol blend. Drivability (cold- or hot-start) of the vehicle decreased noticeably for the 40% butanol blend, especially for the cold-start urban drive cycle. Fuel consumption increased as the blend ratio of butanol increased, due to the lower energy density of butanol compared to diesel. For the highway drive cycle, HC and CO emissions were not significantly impacted but NO_x showed a slight increase as the butanol blend ratio increased. For the steady-state tests, a reduction in filter smoke number with increasing butanol quantity was observed, and an 80% reduction in filter smoke number was observed for the 40% butanol blend.

In addition, n-butanol is also used in CI engines with some new combustion technologies, i.e. HCCI or LTC. These new combustion technologies are the important potential methods to meet the future stringent emission regulations, and the detailed introduction of these combustion technologies have been reviewed by Yao et al. [145].

Liu and Lee [146] investigated the spray and combustion characteristics of pure n-butanol fuel in a constant volume combustion chamber to simulate the diesel engine condition. The ambient temperatures were set to 800, 1000, and 1200 K and the oxygen concentrations were set to 21%, 16%, and 10.5%. All these ambient conditions cover the low temperature combustion and conventional diesel combustion. Results indicated the spray penetration length reduced with the increase of ambient temperature and oxygen concentration, but the oxygen concentration had little effect on the penetration length at high ambient temperature of 1200 K, as shown in Fig. 14. The autoignition delay became longer with decreasing of oxygen concentrations. However, the oxygen concentration had negligible effect on the autoignition timing when it is between 16% and 10.5% at 800–1200 K because a fast evaporation property of n-buatnol ensured the enough combustible mixture at 10.5% oxygen concentration. The flame volume increased as the oxygen concentration decreased. Higher total soot mass and larger soot volume was observable at lower oxygen concentration, as shown in Fig. 15 at 1200 K ambient temperature. However, the soot distribution was not measured at 800 K ambient temperature because a lower ambient temperature resulted in a lower flame

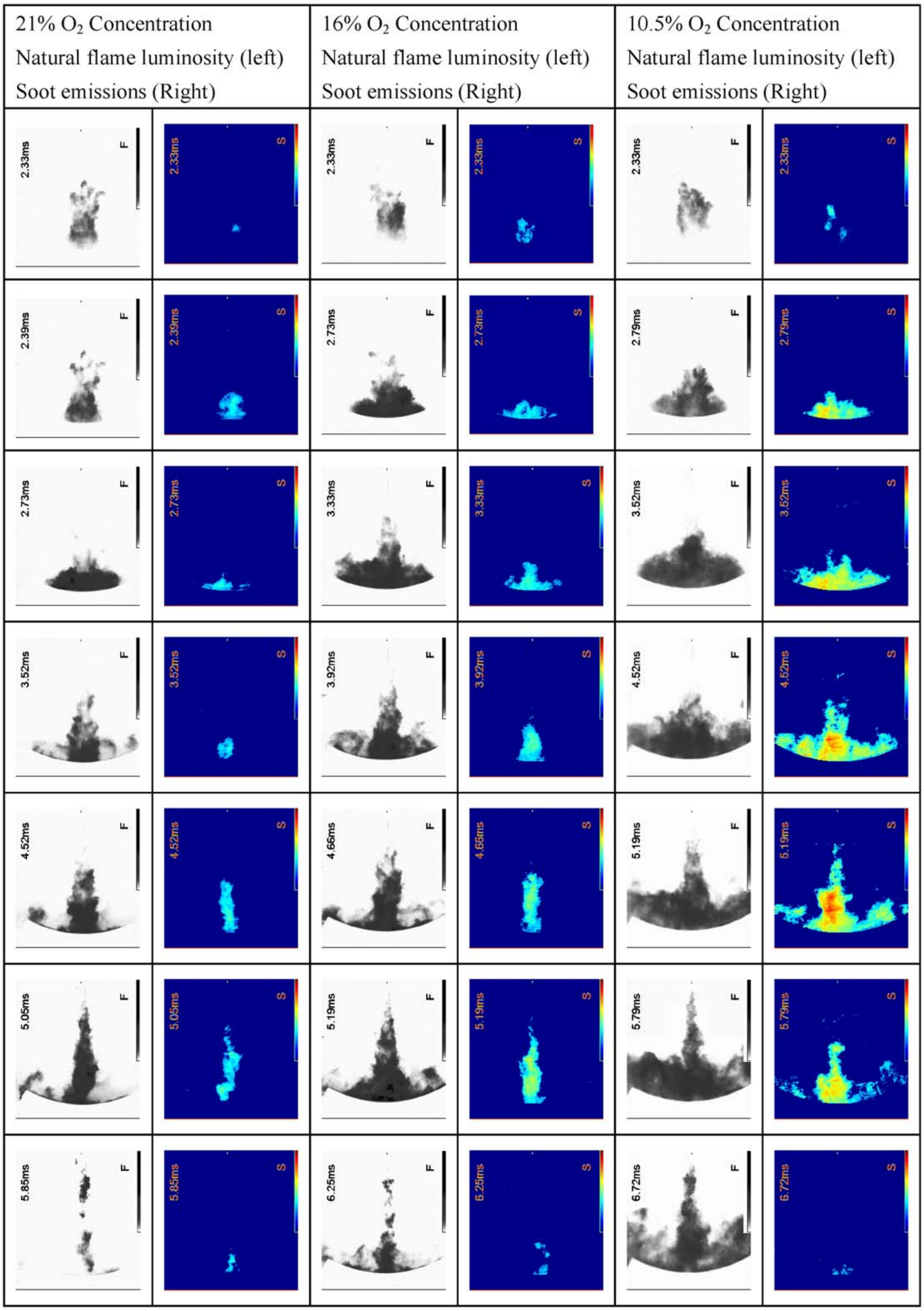


Fig. 15. Natural flame luminosity and soot emission images at 1200 K ambient temperature [146].

temperature and more premixed combustion, inhibiting the soot generation. The total soot emissions at 10.5% oxygen concentration were sensitive to the ambient temperature, and the total soot mass increased dramatically at 1200 K ambient temperature. Therefore, a lower oxygen concentration usually encountered under heavy EGR conditions will increase the difficulty of combustion and soot emissions control even fueling a high oxygenated fuel.

Zhang and Boehman [147] investigated the oxidation of n-butanol and a mixture of n-heptane and n-butanol (containing 60 mol.% of n-heptane and 40 mol.% of n-butanol) in a modified CFR engine at an equivalence ratio of 0.25 and an intake temperature of 120 °C. The engine compression ratio was gradually increased from the lowest point to the point where significant high temperature heat release was observed. Heat release

analysis showed that no noticeable low temperature heat release (LTHR) behavior was observed from the oxidation of neat n-butanol while the n-heptane/n-butanol mixture exhibited pronounced cool flame behavior. The investigation on the oxidation of the mixture of n-heptane/n-butanol showed that the oxidation of n-butanol was facilitated at low temperatures through the radical pool generated from the oxidation of n-heptane.

Saisirirat et al. [148] investigated diesel-HCCI combustion for blended alcohols in diesel-like fuel (n-heptane). Ethanol and n-butanol were blended with n-heptane by varying mole percentage from 0, 18, 37 to 57 and varying dilutions with simulated EGR from 0%, 20% to 40%. The engine speed, intake temperature and equivalence ratio were set at 1500 rpm, 80 °C, and 0.3 respectively. The impacts of alcohols were compared with the primary reference fuels by blending n-heptane with iso-octane at the same molar percentage. The results showed that increasing of ethanol, n-butanol or iso-octane fractions reduce the percentage of LTHR combustion and increase the IMEP moderately. Moreover, the addition of both alcohols in n-heptane has more impact on delaying the heat release rate than that of iso-octane when the blended RON, MON and CN are considered. The similar HCCI combustion were studied by Weiskirch et al. [149] with the neat diesel fuel, volumetric blends of 15%, 30% n-butanol and 85%, 70% diesel, and blends of 7.5% biodiesel, 7.5% ethanol, and 85% diesel. Results demonstrated that the conventional combustion mode is not affected by these blends, except for some advantages of up to 60% reduction in PM-emissions at low air–fuel ratios with the 30% butanol blend. For the HCCI combustion system, butanol blends improve net calorific value corrected fuel consumption as well as emissions of soot and NO_x, but take a penalty of HC and CO emissions compared to the diesel and ethanol–biodiesel–diesel blend fuel.

In addition, there are several studies on the blends of butanol and biodiesel or the blends of butanol, biodiesel, and diesel [143,146,150–152]. The best benefit of these blends is that the disadvantage of higher viscosity for the biodiesel and the lower cetane number for the n-butanol can be offset and more similar properties of diesel can be obtained.

4.3.3. Summary and future directions in CI engine

Based on aforementioned reviews, it can be found that these researches cover some various types of CI engines or similar conditions as CI engines, from the light-duty to the heavy-duty engines, from the CFR engine to the high-pressure constant volume chamber. In addition, some advanced combustion technologies, i.e. HCCI, LTC are also investigated by using the neat n-butanol or n-butanol blends. All these studies reveal some common characteristics:

4.3.3.1. Fuel properties. In comparison to diesohol containing ethanol, the use of butanol in diesohol can solve the problem of phase separation because of more miscibility in the diesel fuel. Further, the blends of diesohol containing butanol have properties close to diesel. A low butanol blends (up to 5 vol.% butanol content) appear to be a good alternative for compression ignition engine utilization without cetane booster additive, and they also allow nearly the same fuel consumption level along with a considerable increase in injector cleanness.

4.3.3.2. Combustion. Compared with the neat diesel fuel, the autoignition is slightly retarded and the premixed combustion peak is higher for the butanol–diesel blends with a low butanol content (<20 vol.%), but there is no appreciable difference in the maximum pressure. In addition, there is no noticeable LTHR behavior from the oxidation of neat n-butanol for the HCCI or LTC combustion process. The autoignition delay of the neat n-butanol becomes longer with the decrease of oxygen concentrations, however, the oxygen concentration has negligible effect on the autoignition delay ranging from 16% to 10.5% at the ambient temperature spanning 800–1200 K.

4.3.3.3. Performance. With increasing percentage of butanol in the blends, an increase in BSFC (~2%) is observed, slightly higher brake thermal efficiency (1–2%) is obtained and lower exhaust temperatures are reached when compared to neat diesel fuel. The impacts of pilot and post injection on engine characteristics by using blended fuels are similar to that found by using pure diesel. But these performance characteristics are measured at a low volumetric blend ratio (<24%). Drivability (cold- or hot-start) of the vehicle decreases noticeably for the 40% butanol blend, especially for the cold-start urban drive cycle.

4.3.3.4. Emissions. The soot is significantly reduced with using the butanol–diesel blends especially at higher load, indicating fuel-bound oxygen has a dominant influence in locally rich zones. Further, there is a larger soot reduction under higher butanol blend ratio. The NO_x emissions are slightly reduced or increased with the use of the butanol–diesel blends compared with the neat diesel fuel, when the butanol blend ratio is below 40%. This is a competitive result between the lower combustion temperature due to butanol's lower heating value and its higher heat of evaporation and the opposing effect of the lower cetane number, and thus longer ignition delay, leading possibly to higher temperatures during the premixed part of combustion. Further, the n-butanol has the further potential to decrease NO_x emissions because the blend fuel allows higher EGR rates without increasing soot emissions. The CO emissions were reduced, but the HC emissions were not affected or slightly increased compared with the neat diesel fuel at a low volumetric blend ratio (<24%). However, the HC and CO emissions increase at the urban drive cycle while they are not significantly impacted at the highway drive cycle at the 40% butanol blend.

Based on these reviews, it can be found that the studies of the n-butanol are limited in diesel engines compared with the abundant research in biodiesel or biodiesel blends. Therefore, more studies on n-butanol are needed in several aspects as following:

1. For the blend fuel properties, some low temperature blend properties need to be studied, i.e. the stability of the butanol–diesel fuels at lower ambient temperatures (−10, −35 °C). And some blend properties of ethanol–butanol–diesel may have the potential to improve the miscibility of ethanol–diesel.
2. Although the studies have shown that a 5% butanol blend is most suitable for the diesel engine, a higher butanol ratio in the blend fuels should be studied from the viewpoints of fuel properties, combustion, performance, emissions, and dependability.
3. There is no report available for the non-regulated emissions of the butanol–diesel fuel, therefore the relevant studies should be enhanced.
4. The control strategies of diesel engines are complicate, i.e. the multiple injection, injection pressure and timing, higher boost, higher EGR ratio, EGR cooling, miller cycle through variable valve timing, etc. All these sophisticated control strategies need to be improved and optimized for the butanol–diesel blends to realize a much higher efficient and cleaner combustion.
5. The new combustion model, HCCI or LTC is one of the main technologies to meet the more stringent emission regulations in the future, therefore more researches are needed on butanol–diesel blends in HCCI or LTC engines.
6. Like in the SI engine, there is few study on the spray and combustion of the butanol using optical diagnostics, although the Liu et al. [153] has shown the results in the chamber using high speed camera and copper vapor laser. However, more optical diagnostics, such as laser introduced fluorescence, laser introduced incandescence, etc. are needed in some more real

diesel engine conditions, i.e. optical engine, rapid compression machine. And these studies are very important to reveal the spray and combustion characteristics in CI engines, and they are also important to the development of CFD models.

5. Conclusions and future research directions

5.1. Summary and conclusions

In this review, the properties of butanol are compared with those of conventional gasoline and diesel fuel and some widely used biofuels, i.e. methanol, ethanol, biodiesel, which indicates that n-butanol has the potential to overcome the drawbacks brought by low-carbon alcohols in several aspects. The main advantages of n-butanol include higher heating value, lower volatility, less ignition problems, good intersolubility with diesel without any cosolvents, more suitable viscosity as a substitute to diesel fuel, a safer fuel to use in high temperatures, easier distribution through existing pipelines, and a longer term benefit for hydrogen. Further, biobutanol also has a number of synergies with bioethanol: production from the same agricultural feedstocks as ethanol, minor changes in fermentation and distillation process as ethanol, facilitating ethanol blending by using the ethanol–gasoline–butanol blends, and assisting in the conversion of vegetable oils into biodiesel like the ethanol. Although the n-butanol has more benefits than the low-carbon alcohols as an engine fuel, there are still some potential problems with the direct use of butanol fuel in the engine, i.e. increasing the fuel-flow due to a lower heating value compared with the gasoline or diesel fuel, incompatible with some fuel system components, a lower octane number than the low-carbon alcohols inhibits the use of higher compression ratio and higher efficiency, and giving a potential aggradation or corrosiveness problem as a substitute for gasoline due to a higher viscosity.

Compared to the biodiesel, the butanol contains more oxygen content, which can reduce the soot emission further, and the NO_x emissions can also be reduced due to its higher heat of evaporation results in a lower combustion temperature. In addition, the viscosity of the butanol is also more suitable for diesel engine. The drawback of n-butanol should be a lower cetane number compared with diesel or biodiesel fuels. However, a lower cetane number may be a need for the diesel fuel in the future to increase autoignition delay and form more premixed combustion, which serves to reduce the soot emissions.

Then, the development of n-butanol production is reviewed, and various methods to increasing butanol production are introduced in detailed. Clostridia can secrete numerous enzymes that facilitate the breakdown of polymetic carbohydrates into monomers for biobutanol production. In a typical ABE fermentation with *C. acetobutylicum*, it can be characterized by two distinct phases acidogenesis and solventogenesis. Genetic modification is a viable method to improve the solvent production and butanol production ratio. The key enzymes and their genes acting on the butanol synthetic pathway in *C. aectobutylcum* are discussed in this review.

Compared to the strain development, the development of fermentation processes has been explored extensively. The major limiting factors associated with ABE fermentation include product toxicity to the producing bacterium, substrate to product conversion efficiency, the ability to utilize an inexpensive cellulosic biomass as a substrate, and the potential for culture degeneration. Productivity in batch reactors is often low due to downtime, long lag phase, and product inhibition. This problem can be eliminated using fed-batch techniques or continuous culture with the application of novel product removal techniques. Product recovery was traditionally performed by distillation. Since butanol has a higher boiling point than water, this procedure uses up a lot of energy and is the reforevery cost-intensive, especially at low butanol concentrations. Other means of recovery i.e. gas stripping, liquid–liquid extraction, and membrane-based processes such as perstraction, reverse osmosis, and pervaporation are also reviewed in Section 3.

In addition, one of the most critical factor not only for butanol production but also for whole bioindustry in securing low price substrates for the processes. To compete with the conventional fossil resource-based chemical industry, the biotechnology industry needs a reliable, cost-effective raw materials infrastructure. Different substracts such as starch-based packaging materials, corn fiber hydrolysate, soy molasses, fruit processing industry waste, and whey permeate were investigated for butanol fermentation. The total solvents produced from these alternative renewable resources ranged from 14.8 to 30.1 g/L. There is no doubt that lignocellulose is potentially the best substrate for butanol production, and more efficient bioconversion of cellulose and hemicellulose is crucial to economic success of the industrial production of butanol. The hydrolysates corn fiber substrate had already been used in the past for biotechnological solvent production in the former Soviet Union.

Next, applications of butanol as a biofuel are summarized from fundamental combustion experiments in some reactors to the substitute for gasoline or diesel fuel in engines. For fundamental combustion experiments, the premixed and non-premixed flames are studied in a wide temperature range from 530 to 1800 K, covering low temperature oxidation and high-temperature oxidation, with the ambient pressures spanning 1–10 atm and the fuel–air equivalence ratios ranging from 0.25 to 2.0. These studies reveal the intermediate species of the butanol in premixed or diffused flames, and also validate the novel chemical kinetics mechanism of the butanol that suggests H-abstraction is the major pathway for n-butanol consumption. The maximum laminar flame velocity of the butanol is approx. 47 cm/s at $\phi = 1.1$, which is higher than that of gasoline, approx. 35 cm/s [154]. The result is consistent with the combustion in the SI engines, in which the butanol has a faster combustion than the gasoline. Although the combustion in SI engines is controlled by the laminar and the turbulent flame speed, the turbulent flame is modeled as a function of the square root of the laminar flame speed meaning that the turbulent flame speed is also faster for the butanol.

In SI engines, studies of the neat n-butanol or n-butanol blends reveal some common characteristics. n-Butanol is similar to PON 87 gasoline in terms of combustion knock characteristics. n-Butanol blends reduce the ignition delay and advance the CAD of 50% MFB compared with the neat gasoline, demonstrating a faster combustion. Therefore, the spark ignition timing should be retarded to obtain maximum break torque when gasoline is replaced by n-butanol. Combustion stability does not vary significantly or slightly improves for n-butanol blends as measured by the COV of IMEP. As the n-butanol concentration is below 20%, there is only a little or no penalty for the specific fuel consumption depending on different combustion control strategies in various SI engines, while the change in specific fuel consumption is within 10% of that of pure gasoline for stoichiometric mixture when n-butanol is up to 40 vol.%. The CO, THC, and NO_x emissions may be reduced or increased depending on the specific engine (i.e. port-injection or direct-injection), operating conditions (i.e. with or without closed-loop air–fuel ratio control, spark timing), and blending ratios. Butanol blends have a higher unburned alcohol emission rates than gasoline and the unburned alcohol emission rates are higher for blends with higher n-butanol content. Aldehyde emissions are higher for the n-butanol blends with formaldehyde as the main constituent, whereas formaldehyde does not increase significantly with addition of ethanol.

In CI engines, studies of the neat n-butanol or n-butanol blends reveal some common characteristics. The use of butanol can solve

the problem of phase separation because of more miscibility in the diesel fuel compared with the ethanol. At low volumetric blending ratio (<24%), the autoignition of butanol–diesel fuel is retarded a little and its premixed combustion peak is higher compared to neat diesel fuel. In addition, there is no noticeable LTHR behavior from the oxidation of neat n-butanol for the HCCI combustion process. As the blend ratio of butanol is below 24%, a little higher specific fuel consumption (~2%) was observed due to a lower heating value compared with neat diesel fuel, whereas the brake thermal efficiency increases a little (1–2%). However, as the blending ratio is up to 40%, drivability of the vehicle decreased noticeably due to the lower cetane number, especially for the cold-start urban drive cycle. Soot is significantly reduced when using the butanol–diesel blends especially at higher load, indicating that fuel-bound oxygen has a dominant influence in locally rich zones. Further, there is further soot reduction under higher butanol blending ratio. As the butanol blending ratio is below 40%, the NO_x emissions are slightly reduced or increased compared with the neat diesel fuel. This is a competitive result between the lower combustion temperature due to butanol's lower heating value and its higher heat of evaporation and the opposing effect of the lower cetane number, and thus longer ignition delay, leading possibly to higher temperatures during the premixed part of combustion. However, n-butanol has the potential to decrease NO_x emissions further with a higher EGR rate because the blend fuel allows higher EGR rates without increasing soot emissions. The CO emission is reduced, but the HC emission is no affected or increased a little compared with neat diesel fuel at lower butanol blend ratio (<24 vol.%). As the butanol blend ratio goes up to 40%, the HC and CO emissions increase at the urban drive cycle while they are not significantly impacted at the highway drive cycle. As a result, the HC and CO emissions are also depending on the specific engine, operating conditions, and blending ratios.

Finally, based on all these reports, it can be concluded that the n-butanol can be used safely and advantageously up to a specific blend ratio with the gasoline or diesel fuel in engines from the viewpoints of similar thermo-physical properties, combustion characteristics, engine performance, and exhaust emissions.

5.2. Future research directions

Although butanol is a better alternative biofuel for the gasoline or diesel engines, applications of butanol are dependent on its production and price. Therefore increasing the production of butanol is much more important in the future. First, it is likely to become a competitive process by systems-level metabolic engineering of strains based on the recent availability of complete genome sequences and new metabolic engineering tools for clostridia. Second, the optimized bioprocess for butanol production could be developed by integrating fermentation and downstream processes compare with strain development. Third, *C. acetobutylicum* can not uptake non-food biomass like cellulose and hemicelluloses effectively, although it contains an apparently complete cellulosome. Scientists' efforts are searching for the genetic mechanism involved in controlling related enzymes in charge of degrading polysaccharides. Instead of studying *C. acetobutylicum*, *S. cerevisiae*, *E. coli* and algae are also regarded as effective biobutanol production systems by the scientists. A new biobutanol industry has begun to emerge with all above research progress.

For the fundamental combustion of n-butanol, there is a need for experimental data to validate conceptual models at a higher equivalence ratio (above 3) and a higher ambient pressure (approx. 40 bar) conditions because these conditions might occur in a diesel engine. In addition, there is also a need for more experimental studies and chemical kinetic models of the blends of butanol and diesel. Finally, researches on the spray and combustion of n-butanol blends are needed in optical engines, rapid compression machine, constant volume chamber, etc., to reveal a more real spray and combustion processes in gasoline or diesel engines. And these optical diagnostic experiments are also important to the development of numerical simulation fueling butanol blends.

For the butanol's applications in SI or CI engines, studies are not as extensive as the case of ethanol or biodiesel. Therefore, more studies of n-butanol are needed in several aspects. First, more extensive studies should be conducted in various engine types, i.e. used engines or new engines, four-stroke or two-stroke engines, port-injection or direct-injection SI engines, common-rail, unit injector, or unit pump CI engines. Second, the control strategies of modern engines are more sophisticated because many advanced technologies are used to reduce emissions and to increase efficiencies, i.e. gasoline direct-injection, multiple injections, boost, EGR and its cooling, variable valve timing and lift, etc. Therefore, it is necessary to understand the behavior of n-butanol in modern engines and to extract maximum benefits from n-butanol through optimized engine design and calibration. Third, more research on emissions should be carried out, including steady and transient measures, regulated and non-regulated emissions, well-to-wheel CO_2 emissions. Further, the reports of trends in THC, CO and NO_x emissions are not consistent, depending on the specific engine, operating conditions, and blending ratios. As a result, studies of the emission mechanisms are needed to reveal the reason why these emissions increase or decrease. Advanced laser diagnostics, i.e. laser introduced fluorescence, laser introduced incandescence, etc. can help to investigate the formation of air–fuel mixture and harmful emissions. These studies will give a more detailed insight on the advantages and disadvantages of butanol blend. Fourth, only few reports can be found for n-butanol or its blends in engines equipped with new combustion techonology, i.e. CAI, HCCI, or LTC. However, these new combustion technologies have the potential to help meet the more stringent emission regulations in the future. Therefore, more researches are needed on butanol or its blends using these new combustion technologies. Last but not least, properties of butanol blends need to be studied, i.e. what water content can be tolerated by the butanol–gasoline blend, whether or not the butanol can improve the miscibility of ethanol–diesel blend like biodiesel.

Acknowledgement

The authors would like to appreciate Xuan Feng from University of Illinois at Urbana-Champaign for his proofreading and English revision of this manuscript. This work was funded by the National Natural Science Found of China (50976078) and the Ministry of Science and Technology of China (2007CB210002). The author Haifeng Liu also thanks the China Scholarship Council for providing a research scholarship in the USA throughout the project [2009]3012.

References

[1] Demirbas A. Political, economic and environmental impacts of biofuels: a review. Appl Energy 2009;86:S108–17.
[2] IEA. Key world energy statistics. Available at: http://www.iea.org/textbase/nppdf/free/2009/key_stats_2009.pdf.
[3] Biofuels 2020, a policy driven logistics and business challenge. Reseach and innovation, position paper 02, 2010. Available at: http://www.dnv.com/binaries/Biofuels%202020%20position%20paper_tcm4-434417.pdfh.
[4] Carbon disclosure project transport report. Analysis based on CDP 2009 data. Available at: http://www.att.com/Common/about_us/files/pdf/Telepresence/CDP_Telepresence_Report_Final.pdf.
[5] Luo L, van der Voet E, Huppes G. Life cycle assessment and life cycle costing of bioethanol from sugarcane in Brazil. Renew Sust Energy Rev 2009;13:1613–9.
[6] Zeng XY, Ma YT, Ma LR. Utilization of straw in biomass energy in China. Renew Sust Energy Rev 2007;11:976–87.

[7] Liu HF, Huang JC, Jin C. The development of the bio-diesel and DME on the diesel engine. Guang Xi Energy Convers 2006;1:28–31.
[8] Murugesan A, Umarani C, Subramanian R, Nedunchezhian N. Bio-diesel as an alternative fuel for diesel engines – a review. Renew Sust Energy Rev 2009;13:653–62.
[9] Murugesan A, Umarani C, Chinnusamy TR, Krishnan M, Subramanian R, Neduzchezhain N. Production and analysis of bio-diesel from non-edible oils – a review. Renew Sust Energy Rev 2009;13:825–34.
[10] Rakopoulos CD, Antonopoulos KA, Rakopoulos DC, Hountalas DT, Giakoumis EG. Comparative performance and emissions study of a direct injection diesel engine using blends of diesel fuel with vegetable oils or bio-diesels of various origins. Energy Convers Manage 2006;47:3272–87.
[11] Qin J, Liu HF, Yao MF, Chen H. Influence of biology oxygenated fuel component on diesel engine characteristics. Trans CSICE 2007;25:281–7.
[12] Qin J, Liu HF, Yao MF, Chen H. Experiment study on diesel engine fueled with biodiesel and diesel fuel. J Combust Sci Technol 2007;13:335–40.
[13] Huang JC, Liu HF, Zhang QC, Zhang FG, Feng GD. The experiment research of the admixture fuel of bio-diesel with diesel on diesel engine. J Guangxi Univ (Nat Sci Ed) 2006;31:185–9.
[14] Fang TG, Lee CF. Bio-diesel effects on combustion processes in an HSDI diesel engine using advanced injection strategies. Proc Combust Inst 2009;32:2785–92.
[15] Fang T, Lin YC, Foong TM, Lee CF. Biodiesel combustion in an optical HSDI diesel engine under low load premixed combustion conditions. Fuel 2009;88:2154–62.
[16] Qi DH, Geng LM, Chen H, Bian YZ, Liu J, Ren XC. Combustion and performance evaluation of a diesel engine fueled with biodiesel produced from soybean crude oil. Renew Energy 2009;34:2706–13.
[17] Bhale PV, Deshpande NV, Thombre SB. Improving the low temperature properties of biodiesel fuel. Renew Energy 2009;34:794–800.
[18] Liu HF, Jin C. The development of ethanol fuels. Guang Xi Energy Conserv 2005;3:31–3.
[19] Liu HF, Zhang QC, Huang H, Huang JC. The application of fuels methanol and dimethyl ether (DME) on engines. Energy Conserv 2006;25:13–6.
[20] Zheng ZQ, YAO MF, Liu HF, Zhang FG, Chen Z. Experimental study on thermo-atmosphere combustion of direct-injection methanol engine. J Combust Sci Technol 2006;12:76–80.
[21] Weber C, Farwick A, Benisch F, Brat D, Dietz H, Subtil T, et al. Trends and challenges in the microbial production of lignocellulosic bioalcohol fuels. Appl Microbiol Biotechnol 2010;87:1303–15.
[22] Luo L, van der Voet E, Huppes G. An energy analysis of ethanol from cellulosic feedstock–corn stover. Renew Sust Energy Rev 2009;13:2003–11.
[23] Hansen AC, Zhang Q, Lyne PWL. Ethanol–diesel fuel blends – a review. Bioresour Technol 2005;96:277–85.
[24] Ezeji T, Qureshi N, Blaschek HP. Production of acetone–butanol–ethanol (ABE) in a continuous flow bioreactor using degermed corn and *Clostridium beijerinckii*. Process Biochem 2007;42:34–9.
[25] Hansen AC, Kyritsis DC, Lee CF. Characteristics of biofuels and renewable fuel standards. In: Vertes AA, Blaschek HP, Yukawa H, Qureshi N, editors. Biomass to biofuels – strategies for global industries. New York: John Wiley; 2009.
[26] Hansen AC, Gratton MR, Yuan W. Diesel engine performance and NO_x emissions from oxygenated biofuels and blends with diesel fuel. Trans ASABE 2006;9:589–95.
[27] Li DG, Huang Z, Lŭ XC, Zhang WG, Yang JG. Physico-chemical properties of ethanol–diesel blend fuel and its effect on performance and emissions of diesel engines. Renew Energy 2005;30:67–76.
[28] Simonsen H, Chomiak J. Testing and evaluation of ignition improvers for ethanol in a DI diesel engine. SAE 952512; 1995.
[29] Togbe C, Dayma G, Mze-Ahmed A, Dagaut P. Experimental and modeling study of the kinetics of oxidation of simple biodiesel-biobutanol surrogates: methyl octanoate-butanol mixtures. Energy Fuel 2010;24:3906–16.
[30] Fortman JL, Chhabra S, Mukhopadhyay A, Chou H, Lee TS, Steen E, et al. Biofuel alternatives to ethanol: pumping the microbial well. Trends Biotechnol 2008;26:375–81.
[31] Ezejia TC, Qureshib N, Blascheka HP. Continuous butanol fermentation and feed starch retrogradation: butanol fermentation sustainability using *Clostridium beijerinckii* BA101. J Biotechnol 2005;115:179–87.
[32] Rakopoulos DC, Rakopoulos CD, Giakoumis EG, Dimaratos AM, Kyritsis DC. Effects of butanol–diesel fuel blends on the performance and emissions of a high-speed DI diesel engine. Energy Convers Manage 2010;51: 1989–97.
[33] Wallner T, Miers SA, McConnell S. A comparison of ethanol and butanol as oxygenates using a direct-injection, spark-ignition engine. J Eng Gas Turb Power 2009;131:032802.1–9.
[34] Freeman J, Williams J, Minner S, Baxter C, DeJovine J, Gibbs L, et al. Alcohols and ethers: a technical assessment of their application as fuels and fuel components, API publication 4261. 2nd ed. New York: American Institute of Physics; 1988.
[35] Dean JA. Lange's handbook of chemistry. 14th ed. New York: McGraw-Hill; 1992.
[36] BP DuPont fact sheet biobutanol. Available at: http://www2.dupont.com/Production_Agriculture/en_US/assets/downloads/pdfs/BP_DuPont_Fact_Sheet_Biobutanol.pdf.
[37] Zhang LY, Yang Y, Shi ZP. Performance optimization of property-improved biodiesel manufacturing process coupled with butanol extractive fermentation. Chin J Biotechnol 2008;24:1943–8.
[38] Volesky B, Szczesny T. Bacterial conversion of pentose sugars to acetone and butanol. Adv Biochem Eng/Biotechnol 1983;27:101–18.
[39] Weizmann C. British Patent 1912; p. 4845.
[40] Weizmann C. U.S. Patent 1916; p. 1315585.
[41] Dürre P. Fermentative butanol production: bulk chemical and biofuel. Ann N Y Acad Sci 2008;1125:353–62.
[42] https://www.biofuelsdatabase.org/wiki/index.php5/N-Butanol_Pathway.
[43] Mays T. DuPont and BP disclose advanced biofuels partnership targeting multiple butanol molecules. Available at: http://www2.dupont.com/EMEA_Media/en_GB/newsreleases_2008/article20080214.html. Accessed 14, Feb, 2008.
[44] Kharkwal S, Karimi IA, Chang MW, Lee DY. Strain improvement and process development for biobutanol production. Recent Pat Biotechnol 2009;3:202–10.
[45] Dürre P. Handbook on clostridia. CRC Press; 2005. p. 659–699.
[46] Paredes CJ, Alsaker KV, Papoutsakis ET. A comparative genomic view of clostridial sporulation and physiology. Nat Microbiol Rev 2005;3:969–78.
[47] Harris LM, Welker NE, Papoutsakis ET. Northern, morphological, and fermentation analysis of *spo0A* inactivation and overexpression in *Clostridium acetobutylicum* ATCC 824. J Bacteriol 2002;184:3586–97.
[48] Lee SY, Park HJ, Nielsen LK, Kim J, Jung KS. Fermentative butanol production by clostridia. Biotechnol Bioeng 2008;101:209–28.
[49] Ezeji TC, Qureshi N, Blaschek HP. Butanol fermentation research: upstream and downstream manipulations. Chem Rec 2004;4:305–14.
[50] Qureshi N, Blaschek HP. Evaluation of recent advances in butanol fermentation, upstream, and downstream processing. Bioprocess Biosyst Eng 2001;24:219–26.
[51] Lépiz-Aguilar L, Rodríguez-Rodríguez CE, Arias ML, Lutz G, Ulate W. Butanol production by *Clostridium beijerinckii* BA101 using cassava flour as fermentation substrate: enzymatic versus chemical pretreatments. World J Microbiol Biotechnol 2011, doi:10.1007/s11274-010-0630-1.
[52] Jesse TW, Ezeji TC, Qureshi N, Blaschek HP. Production of butanol from starch-based waste packing peanuts and agricultural waste. J Ind Microbiol Biotechnol 2002;29:117–23.
[53] Ebener J, Qureshi N, Blaschek HP. Corn fiber hydrolysis and rermentation to butanol using *Clostridium beijerinckii* BA101. In: 25th symposium on biotechnology for fuels and chemicals. 2003.
[54] Qureshi N, Lolas A, Blaschek HP. Soy molasses as fermentation substrate for production of butanol using *Clostridium beijerinckii* BA101. J Ind Microbiol Biotechnol 2001;26:290–5.
[55] Maddox IS, Qureshi N, Gutierrez NA. In: Woods DR, editor. In the clostridia and biotechnology. Boston: Butterworth–Heinemann; 1993. p. 343–69.
[56] Woods DR. The genetic engineering of microbial solvent production. Trends Biotechnol 1995;113:259–64.
[57] Bowles LK, Ellefson WL. Effects of butanol on *Clostridium acetobutylicum*. Appl Environ Microbiol 1985;50:1165–70.
[58] Jones DT, Woods DR. Acetone–butanol fermentation revisited. Microbiol Rev 1986;50:484–524.
[59] Cary JW, Petersen DJ, Papoutsakis ET, Bennett GN. Cloning and expression of *Clostridium acetobutylicum* ATCC 824 acetoacetyl-coenzyme A: acetate/butyrate: coenzyme a-transferase in *Escherichia coli*. Appl Environ Microbiol 1990;56: 1576–83.
[60] Gerischer U, Dürre P. Cloning, sequencing, and molecular analysis of the acetoacetate decarboxylase gene region from *Clostridium acetobutylicum*. J Bacteriol 1990;172:6907–18.
[61] Petersen DJ, Bennett GN. Purification of acetoacetate decarboxylase from *Clostridium acetobutylicum* ATCC 824 and cloning of the acetoacetate decarboxylase gene in *Escherichia coli*. Appl Environ Microbiol 1990;56:3491–8.
[62] Mermelstein LD, Papoutsakis ET. In vivo methylation in *Escherichia coli* by the *Bacillus subtilis* phage 43T methyltransferase to protect plasmids from restriction upon transformation of *Clostridium acetobutylicum* ATCC 824. Appl Environ Microbiol 1993;59:1077–81.
[63] Nair RV, Papoutsakis ET. Expression of plasmid-encoded *aad* in *Clostridium acetobutylicum* M5 restores vigorous butanol production. J Bacteriol 1994;176:5843–6.
[64] Harris LM, Blank L, Desai RP, Welker NE, Papoutsakis ET. Fermentation characterization and flux analysis of recombinant strains of *Clostridium acetobutylicum* with an inactivated *solR* gene. J Ind Microbiol Biotechnol 2001;27:322–8.
[65] Nair RV, Green EM, Watson DE, Bennett GN, Papoutsakis ET. Regulation of the *sol* locus genes for butanol and acetone formation in *Clostridium acetobutylicum* ATCC 824 by a putative transcriptional repressor. J Bacteriol 1999;181:319–30.
[66] Thormann K, Feustel L, Lorenz K, Nakotte S, Dürre P. Control of butanol formation in *Clostridium acetobutylicum* by transcriptional activation. J Bacteriol 2002;184:1966–73.
[67] Scotcher MC, Bennett GN. SpoIIE regulates sporulation but does not directly affect solventogenesis in *Clostridium acetobutylicum* ATCC 824. J Bacteriol 2005;187:1930–6.
[68] Desai RP, Papoutsakis ET. Antisense RNA strategies formetabolic engineering of *Clostridium acetobutylicum*. Appl Environ Microbiol 1999;65:936–45.
[69] Tummala SB, Welker NE, Papoutsakis ET. Design of antisense RNA constructs for downregulation of the acetone formation pathway of *Clostridiuma cetobutylicum*. J Bacteriol 2003;185:1923–34.
[70] Tomas C, Beamish J, Papoutsakis ET. Transcription analysis of butanol stress and tolerance in *Clostridium acetobutylicum*. J Bacteriol 2004;186:2006–18.

[71] Borden JR, Papoutsakis ET. Dynamics of genomic-library enrichment and identification of solvent tolerance genes for *Clostridium acetobutylicum*. Appl Environ Microbiol 2007;73:3061–8.
[72] Alper H, Moxley J, Nevoigt E, Fink GR, Stephanopoulos G. Engineering yeast transcription machinery for improved ethanol tolerance and production. Science 2006;314:1565–8.
[73] Mermelstein LD, Papoutsakis ET, Petersen DJ, Bennett GN. Metabolic engineering of *Clostridium acetobutylicum* ATCC 824 for increased solvent production by enhancement of acetone formation enzyme-activities using a synthetic acetone operon. Biotechnol Bioeng 1993;42:1053–60.
[74] Nair RV, Bennett GN, Papoutsakis ET. Molecular characterization of an aldehyde/alcohol dehydrogenase gene from *Clostridium acetobutylicum* ATCC 824. J Bacteriol 1994;176:871–85.
[75] Green EM, Boynton ZL, Harris LM, Rudolph FB, Papoutsakis ET, Bennett GN. Genetic manipulation of acid formation pathways by gene inactivation in *Clostridium acetobutylicum* ATCC 824. Microbiology (UK) 1996;142:2079–86.
[76] Shao LJ, Hu SY, Yang Y, Gu Y, Chen J, Sheng Y, et al. Targeted gene disruption by use of a group II intron (targetron) vector in *Clostridium acetobutylicum*. Cell Res 2007;17:963–5.
[77] Harris LM, Desai RP, Welker NE, Papoutsakis ET. Characterization of recombinant strains of the *Clostridium acetobutylicum* butyrate kinasein activation mutant: need for new phenomenological models for solven to genesis and butanol inhibition. Biotechnol Bioeng 2000;67:1–11.
[78] Harris LM, Blank L, Desai RP, Welker NE, Papoutsakis ET. Fermentation characterization and flux analysis of recombinant strains of *Clostridium acerobutylicurn* with an inactivated *solR* gene. J Ind Microbiol Biotechnol 2001;27:322–8.
[79] Tummala SB, Papoutsakis ET, Junne SG. Antisense RNA down-regulation of coenzyme A transferase combined with alcohol-aldehyde dehydrogenase overexpression leads to predominantly alcohologenic *Clostridium acetobutylicum* fermentations. J Bacteriol 2003;185:3644–53.
[80] Tomas CA, Welker NE, Papoutsakis ET. Overexpression of *groESL* in *Clostridium acetobutylicum* results in increased solvent production and tolerance, prolonged metabolism, and changes in the cell's transcriptional program. Appl Environ Microbiol 2003;69:4951–65.
[81] Jiang Y, Xu CM, Dong F, Yang Y, Jing W, Yang S. Disruption of the acetoacetate decarboxylase gene in solvent-producing *Clostridium acetobutylicum* increases the butanol ratio. Metab Eng 2009;11:284–91.
[82] Ezeji TC, Qureshi N, Blaschek HP. Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. Appl Microbiol Biotechnol 2004;63:653–8.
[83] Formanek J, Mackie R, Blaschek HP. Enhanced butanol production by *Clostridium beijerinckii* BA101 grown in semidefined P2 medium containing 6 percent maltodextrin or glucose. Appl Environ Microbiol 1997;63:2306–10.
[84] Sun ZH, Ni Y. Recent progress on industrial fermentative production of acetone-butanol-ethanol by *Clostridium acetobutylicum* in China. Appl Microbiol Biotechnol 2009;83:415–23.
[85] Qureshi N, Schiripsema J, Lienharde J, Blaschek HP. Continuous solvent production by *Clostridium beijerinckii* BA101 immobilized by adsorption onto brick. World J Microbiol Biotechnol 2000;16:377–82.
[86] Ezeji TC, Qureshi N, Blaschek HP. Bioproduction of butanol from biomass: from genes to bioreactors. Curr Opin Biotech 2007;18:220–7.
[87] Matsumura M, Kataoka H, Sueki M, Araki K. Energy saving effect of pervaporation using oleyl alcohol liquid membrane in butanol purification. Bioproc Eng 1988;3:93–100.
[88] Qureshi N, Blaschek HP. Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation. Biotechnol Prog 1999;15:594–602.
[89] Qureshi N, Blaschek HP. Butanol production using *Clostridium beijerinckii* BA101 hyper-butanol producing mutant strain and recovery by pervaporation. Appl Biochem Biotechnol 2000;84:225–35.
[90] Qureshi N, Meagher MM, Hutkins RW. Recovery of butanol from model solutions and fermentation broth using a silicalite/silicone membrane. J Membr Sci 1999;158:115–25.
[91] Izák P, Schwarz K, Ruth W, Bahl H, Kragl U. Increased productivity of *Clostriduim acetobutylicum* fermentation of acetone, butanol, and ethanol by pervaporation through supported ionic liquid membrane. Appl Microbiol Biotechnol 2008;78:597–602.
[92] Ezeji TC, Qureshi N, Blaschek HP. Bioproduction of butanol from biomass: from genes to bioreactors. Curr Opin Biotechnol 2007;18:220–7.
[93] Zheng YN, Li LZ, Xian M, Ma YJ, Yang JM, Xu X, et al. Problems with the microbial production of butanol. Microbiol Biotechnol 2009;36:1127–38.
[94] Evans PJ, Wang HY. Enhancement of butanol formation by *Clostridium acetobutylicum* in the presence of decanol-oleyl alcohol mixed extractants. Appl Environ Microbiol 1988;54:1662–7.
[95] Blaschek HP, Formanedk J, Chen CK. Patent No. 6358717; 2002.
[96] Parekh M, Formanek J, Blaschek HP. Pilot-scale production of butanol by *Clostridium beijerinckii* BA101 using a low-cost fermentation medium based on corn steep water. Appl Environ Microbiol 1999;51:152–7.
[97] Qureshi N, Blaschek HP. Economics of butanol fermentation using hyper-butanol producing *Clostridium beijerinckii* BA101. Food Bioprod Process 2000;78:139–44.
[98] Liu ZY, Ying Y, Li FL, Ma CQ, Xu P. Butanol production by *Clostridium beijerinckii* ATCC 55025 from wheat bran. J Ind Microbiol Biotechnol 2010;37:495–501.
[99] Ezeji TC, Qureshi N, Blaschek HP. Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation. Biotechnol Bioeng 2007;97:1460–9.
[100] Ezeji TC, Blaschek HP. Fermentation of dried distillers' grains and solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic clostridia. Biores Technol 2008;99:5232–42.
[101] Ezeji TC, Qureshi N, Blaschek HP. Production of acetone butanol (AB) from liquefied corn starch, a commercial substrate, using *Clostridium beijerinckii* coupled with product recovery by gas stripping. J Ind Microbiol Biotechnol 2007;34:771–7.
[102] Zverlov VV, Berezina O, Velikodvorskaya GA, Schwarz WH. Bacteria acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery. Appl Microbiol Biotechnol 2006;71:587–97.
[103] Atsumi S, Liao JC. Metabolic engineering for advanced biofuels production from *Escherichia coli*. Curr Opin Biotechnol 2008;19:414–9.
[104] Inui M, Suda M, Kimura S, Suda M, Yasuda K, Suzuki H, et al. Expression of *Clostridium acetobutylicum* butanol synthetic genes in *Escherichia coli*. Appl Microbiol Biotechnol 2008;77:1305–16.
[105] Atsumi S, Hanai T, Liao JC. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuel. Nature 2008;451:86–9.
[106] Steen EJ, Chan R, Prasad N, Myers S, Petzold CJ, Redding A, et al. Metabolic engineering of *Saccharomyces cerevisiae* for the production of n-butanol. Microbe Cell Fact 2008;7:36–43.
[107] Biofuels international. Available at: http://www.biofuels-news.com/content_item_details.php?item_id=389.
[108] Barnard JA. Trans Faraday Soc 1957;53(11):1423–30.
[109] Roberts AF. J Imp Coll Chem Eng Soc 1959;12:58–73.
[110] McEnally CS, Pfefferle LD. Fuel decomposition and hydrocarbon growth processes for oxygenated hydrocarbons: butyl alcohols. Proc Combust Inst 2005;30:1363–70.
[111] Yang B, Oswald P, Li Y, Wang J, Wei L, Tian Z, et al. Identification of combustion intermediates in isomeric fuel-rich premixed butanol–oxygen flames at low pressure. Combust Flame 2007;148:198–209.
[112] Dagaut P, Sarathy SM, Thomson MJ. A chemical kinetic study of n-butanol oxidation at elevated pressure in a jet stirred reactor. Proc Combust Inst 2009;32:229–37.
[113] Dagaut P, Togbé C. Oxidation kinetics of butanol–gasoline surrogate mixtures in a jet-stirred reactor: experimental and modeling study. Fuel 2008;87:3313–21.
[114] Sarathy SM, Thomson MJ, Togbé C, Dagaut P, Halter F, Mounaim-Rousselle C. An experimental and kinetic modeling study of n-butanol combustion. Combust Flame 2009;156:852–64.
[115] Moss JT, Berkovitz AM, Oehlschlaeger MA, Biet J, Warth V, Glaude P, et al. An experimental and kinetic modelling study of the oxidation of the four isomers of butanol. J Phys Chem A 2008;112:10843–55.
[116] Black G, Curran HJ, Pichon S, Simmie JM, Zhukov V. Bio-butanol: combustion properties and detailed chemical kinetic model. Combust Flame 2010;157:363–73.
[117] Gu XL, Huang ZH, Li QQ, Tang CL. Measurements of laminar burning velocities and markstein lengths of n-butanol-air premixed mixtures at elevated temperatures and pressures. Energy Fuel 2009;23:4900–7.
[118] Dagaut P, Togbé C. Experimental and modeling study of the kinetics of oxidation of butanol-n-heptane mixtures in a jet-stirred reactor. Energy Fuel 2009;23:3527–35.
[119] Beeckmann J, Röhl O, Peters N. Numerical and experimental investigation of laminar burning velocities of iso-octane, ethanol and n-butanol. SAE 2009-01-2784; 2009.
[120] Veloo PS, Wang YL, Egolfopoulos FN, Westbrook CK. A comparative experimental and computational study of methanol, ethanol, and n-butanol flames. Combust Flame 2010;157:1989–94.
[121] Yacoub Y, Bara R, Gautam M. The performance and emission characteristics of C1-C5 alcohol–gasoline blends with matched oxygen content in a single cylinder spark ignition engine. Proc Inst Mech Eng A – Power Energy 1998;212:363–79.
[122] Gautam M, Martin DW. Combustion characteristics of higher-alcohol/gasoline blends. Proc Inst Mech Eng 2000;214A:497–511.
[123] Gautam M, Martin DW. Emission characteristics of higher-alcohol/gasoline blends. Proc Inst Mech Eng 2000;214A:165–82.
[124] Szwaja S, Naber JD. Combustion of n-butanol in a spark-ignition IC engine. Fuel 2010;89:1573–82.
[125] Alasfour FN. Butanol – a single cylinder engine study: availability analysis. Appl Therm Eng 1997;17:537–49.
[126] Dernotte J, Mounaim-Rousselle C, Halter F, Seers P. Evaluation of butanol-gasoline blends in a port fuel-injection, spark-ignition engine. Oil Gas Sci Technol – Rev IFP 2010;65:345–51.
[127] Wallner T, Frazee R. Study of regulated and non-regulated emissions from combustion of gasoline, alcohol fuels and their blends in a DI-SI engine. SAE 2010-01-1571; 2010.
[128] Yang J, Yang XL, Liu JP, Han ZY, Zhong ZH. Dyno test investigations of gasoline engine fueled with butanol-gasoline blends. SAE 2009-01-1891; 2009.
[129] Cairns A, Stansfield P, Fraser N, Blaxill H, Gold M, Rogerson J, et al. A study of gasoline-alcohol blended fuels in an advanced turbocharged DISI engine. SAE 2009-01-0138; 2009.

[130] Cairns A, Todd A, Aleiferis P, Fraser N, Malcolm J. A study of alcohol blended fuels in an unthrottled single cylinder spark ignition engine. SAE 2010-01-0618; 2010.
[131] Williams J, Goodfellow C, Lance D, Ota A, Nakata K, Kawatake K, et al. Impact of butanol and other bio-components on the thermal efficiency of prototype and conventional engines. SAE 2009-01-1908; 2009.
[132] Aleiferis P, Pereira JS, Richardson D, Wallace S. Characteristics of ethanol, butanol, iso-octane and gasoline sprays and combustion from a multi-hole injector in a DISI engine. SAE 2008-01-1591; 2008.
[133] Waterland LR, Venkatesh S, Unnasch S. Safety and performance assessment of ethanol/diesel blends (E-Diesel). National Renewable Energy Laboratory – Subcontractor Report. NREL/SR-540-34817; 2003.
[134] Qi DH, Chen H, Lee CF, Geng LM, Bian YZ. Experimental studies of a naturally aspirated, DI diesel engine fuelled with ethanol-biodiesel-water microemulsions. Energy Fuel 2010;24:652–63.
[135] Qi DH, Chen H, Matthews RD, Bian YZ. Combustion and emission characteristics of ethanol–biodiesel–water micro-emulsions used in a direct injection compression ignition engine. Fuel 2010;89:958–64.
[136] Qi DH, Chen H, Lee CF, Geng LM, Bian YZ, Ren XC. Performance and combustion characteristics of biodiesel–diesel–methanol blend fuelled engine. Appl Energy 2010;87:1679–86.
[137] Yuan W, Hansen AC, Zhang Q. Vapor pressure and normal boiling point predictions for pure methyl esters and biodiesel fuels. Fuel 2005;84: 943–50.
[138] Chotwichien A, Luengnaruemitchai A, Jai-In S. Utilization of palm oil alkyl esters as an additive in ethanol–diesel and butanol–diesel blends. Fuel 2009;88:1618–24.
[139] Zoldy M, Hollo A, Thernesz A. Butanol as a diesel extender option for internal combustion engines. SAE 2010-01-0481; 2010.
[140] Yao MF, Wang H, Zheng ZQ, Yue Y. Experimental study of n-butanol additive and multi-injection on HD diesel engine performance and emissions. Fuel 2010;89:2191–201.
[141] Yao MF, Zhang QC, Liu HF, Zheng ZQ, Zhang P, Lin ZQ, et al. Diesel engine combustion control: medium or heavy EGR. SAE 2010-01-1125; 2010.
[142] Rakopoulos DC, Rakopoulos CD, Hountalas DT, Kakaras EC, Giakoumis EG, Papagiannakis RG. Investigation of the performance and emissions of bus engine operating on butanol/diesel fuel blends. Fuel 2010;89: 2781–90.
[143] Liu Y, Cheng WL, Lee CF, Li J. A study of the effects of volatility on butanol-biodiesel-diesel spray and combustion. Central States Section of the Combustion Institute; 2010.
[144] Miers SA, Carlson RW, McConnell SS, Ng HK, Wallner T, Esper JL. Drive cycle analysis of butanol/diesel blends in a light-duty vehicle. SAE 2008-01-2381; 2008.
[145] Yao MF, Zheng ZL, Liu HF. Progress and recent trends in homogeneous charge compression ignition (HCCI) engines. Prog Energy Combust Sci 2009;35:398–437.
[146] Liu HF, Lee CF. Spray and combustion in a constant volume combustion chamber with some biofuels. In: Graduate automotive technology education (GATE) seminar: automotive bio-energy, September 29. 2010.
[147] Zhang Y, Boehman AL. Oxidation of 1-butanol and a mixture of n-heptane/1-butanol in a motored engine. Combust Flame 2010;157:1816–24.
[148] Saisirirat P, Foucher F, Chanchaona S, Mounaïm-Rousselle C. Effects of ethanol, n-butanol–n-heptane blended on low temperature heat release and HRR phasing in diesel-HCCI. SAE Paper 2009-24-0094; 2009.
[149] Weiskirch C, Kaack M, Blei I, Eilts P. Alternative fuels for alternative and conventional diesel combustion systems. SAE 2008-01-2507; 2008.
[150] Liu Y, Cheng WL, Huo M, Lee CF, Li J. Effects of micro-explosion on butanol-biodiesel-diesel spray and combustion. In: ILASS-Americas 22nd annual conference on liquid atomization and spray systems. 2010.
[151] Laza T, Kecskés R, Bereczky Á, Penninger A. Examination of burning processes of regenerative liquid fuel and alcohol mixtures in diesel engine. Periodica Polytechnica Ser Mech Eng 2006;50:11–26.
[152] Liu HF, Lee CF, Huo M, Yao MF. Comparison of ethanol and butanol as additives in soybean biodiesel using a constant volume combustion chamber. Energy Fuels 2011;25:1837–46.
[153] Liu HF, Lee CF, Huo M, Yao MF. Combustion characteristics and soot distributions of neat butanol and neat soybean biodiesel. Energy Fuels 2011, doi:10.1021/ef1017412.
[154] Heywood JB. Internal combustion engine fundamentals. New York: McGraw-Hill; 1988.